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CONTENTS

ANIMAL PHYSIOLOGY AND BIOCHEMISTRY

ANIMAL BREEDING

BOOK REVIEWS

Acta Agronomica Hungarica, Vol. 39 (1–2), pp. 3–10 (1990)

Soil science and agrochemistry

DID THE RADIOACTIVE POLLUTION – IN CONSEQUENCE OF THE DISASTER AT THE CHERNOBYL NUCLEAR FACILITY – HAVE A POSITIVE INFLUENCE ON THE PLANTS IN HUNGARY?

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(Received: 14 December 1987; accepted in revised form 18 February 1988)

Due to the accident at the Chernobyl nuclear power plant (26.04.1986. USSR) the radioactive contamination level; e.g. ^{131}I, ^{134}Cs, ^{137}Cs of the soil-plant-animal-man biological-chain increased significantly in Hungary, as in other European countries.

The external dose burden from the contaminated ground-surface and atmosphere, and the radioactive isotopes taken up mostly directly through the leaves had a low-dose effect on the plants, which was in the dose-range of stimulation (biopositive effect).

Keywords: Chernobyl, contamination, low-dose, nuclear accident, radioactive isotopes, radiostimulation

Introduction

There are various natural radioisotopes – e.g. ^{40}K, ^{226}Ra – which cause (connected with the cosmic radiation) the natural background radiation level in the biological chain; but in consequence of the nuclear weapon tests the biosphere has been contaminated also with artificial radioisotopes – e.g. ^{89}Sr, ^{90}Sr, ^{131}I, ^{134}Cs, ^{137}Cs, ^{140}Ba – mainly with fission products. In the last 2 decades, till 1985 (after the Moscow atom-stop agreement, 1963) the natural radiation level was much higher than the artificial one in Hungary, and generally throughout Europe.

However in 1986 – due to the disaster at the Chernobyl nuclear facility – the radioactive contamination increased significantly, also in the countries rather far from Chernobyl (Láng, 1986) (Szabó, 1986), (Sieker, 1986), (Deworm, 1987).

Radioactive pollution and radiation dose

Figures 1 and 2 show the ground surface contamination, corrected for May, 1986, and the time dependence of the ground surface contamination due to the measured isotopes (Biró et al., 1986).

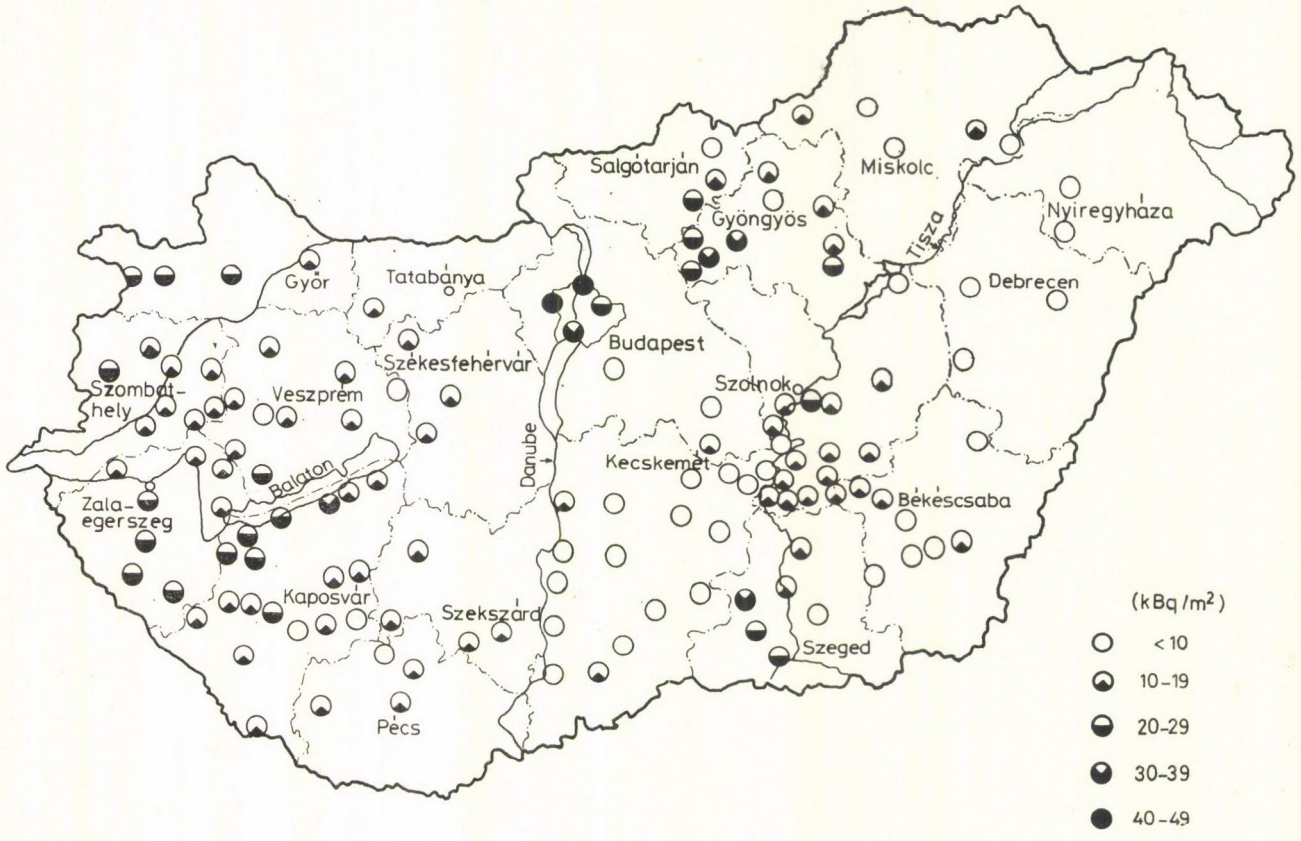

Fig. 1. **Ground surface contamination corrected for 15 May 1986 measured at different locations in Hungary (Biró et al., 1986)**

An important conclusion, that the ground surface pollution — as a function of the different meteorological conditions — could vary by a factor of 2 even within distances of only several kilometres. Among different regions of Hungary much larger variations were found. The ground surface contamination in Hungary had been the highest in the northern and western parts, as one might expect from the fall-out distribution. In other parts of the country the contamination values were lower by nearly an order of magnitude.

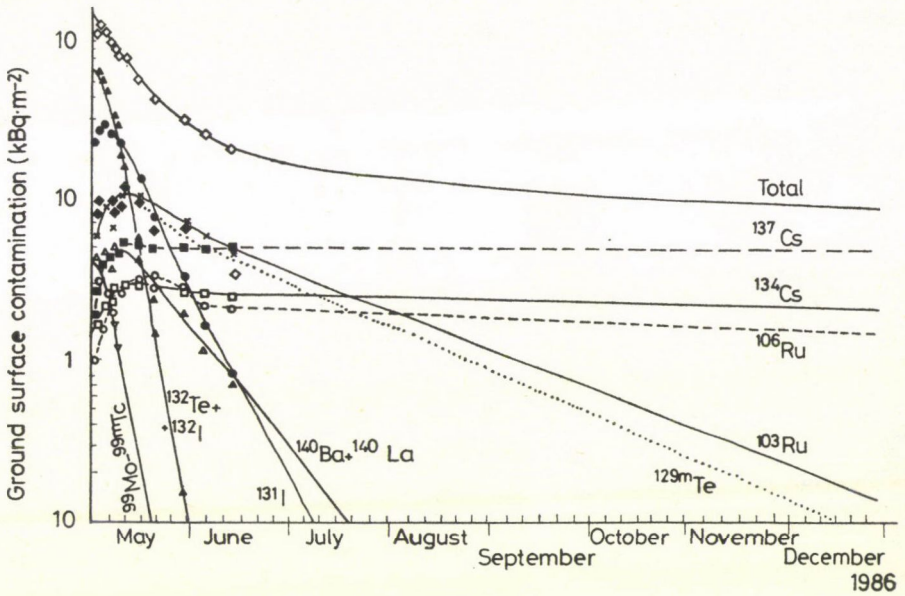

Fig. 2. Ground surface contamination measured by in situ gamma spectroscopy in the CRIP (from 30 April to 12 June 1986) and the extrapolated values calculated to 31 December 1986 (Biró et al., 1986)

The dose rate contributions of the individual isotopes, originating from the Chernobyl accident were derived from the *in situ* gamma spectrometry and the data are shown in Table 1 (Andrási et al., 1986).

The excess gamma dose rates, measured in the Central Research Institute for Physics are shown in Fig. 3 (Láng, 1986).

In view of the surface pollution measurements, Budapest and some of the northern countries (Heves, Borsod) were to be considered the most contaminated areas in Hungary, with a ground surface contamination in the range of 25/50 kBq/m² (Biró et al., 1986). In the first 2 weeks of May, 1986, the ground surface contamination ranged from 10 to 30 kBq/m² in the western and south-western counties; whereas the least polluted areas were in the south-eastern and eastern parts of the country.

Table 1

Dose rate (nGy/h) calculated from the ground contamination measured by in situ gamma spectroscopy in the CRIP and the extrapolated values calculated to 31, December 1986 (Andrási et al., 1986)

Isotope / Date	Measured values											Extrapolated values		
	04.30	05.01	05.03	05.05	05.06	05.08	05.09	05.14	05.19	05.29	06.04	07.31	09.30	12.31
^{95}Zr	—	0.60	0.81	0.33	0.59	0.66	0.37	0.67	0.37	0.34	0.32	0.2	0.1	0.03
^{95}Nb	—	0.78	0.51	0.77	0.77	0.84	0.75	1.0	1.3	0.86	0.60	0.3	0.2	0.06
^{99}Mo-^{99m}Tc	3.3	3.4	2.1	2.1	0.4	1.2	—	—	—	—	—	—	—	—
^{103}Ru	12.4	15.3	18.8	19.1	14.6	21.0	22.9	21.6	18.2	15.2	12.4	4.6	1.8	0.31
^{106}Ru	0.9	2.7	1.4	2.2	1.7	2.5	2.3	2.6	2.9	2.8	1.9	1.7	1.6	1.3
^{110m}Ag	—	—	—	—	1.1	—	1.1	0.6	0.8	0.9	0.9	0.8	0.7	0.5
^{129m}Te	2.1	2.4	2.3	2.5	2.1	2.2	2.8	2.5	1.7	1.7	1.3	0.4	0.13	0.017
^{131}I	36.5	43.1	46.5	41.2	40.7	37.0	32.5	22.3	13.2	5.6	2.9	0.05	—	—
^{132}Te	24.0	22.5	18.7	12.9	7.7	6.2	2.2	0.9	0.06	—	—	—	—	—
^{132}I	268	244	202	138	126	81.9	68.1	24.3	6.6	0.8	—	—	—	—
^{133}I	2.9	1.6	0.56	—	—	—	—	—	—	—	—	—	—	—
^{134}Cs	11.8	11.8	15.4	16.0	19.2	18.6	20.2	20.7	19.2	18.7	17.9	17.0	16.2	14.8
^{136}Cs	5.7	5.4	7.7	6.2	7.9	5.7	7.1	4.4	3.4	1.5	1.6	0.08	0.005	—
^{137}Cs	4.6	6.7	10.2	11.2	11.5	11.3	13.4	12.9	12.4	12.6	12.5	12.5	12.4	12.3
^{140}Ba-^{140}La	24.7	23.5	26.5	22.1	29.0	26.7	32.6	25.0	15.3	11.8	7.0	0.6	0.02	—
Total	397	384	354	275	267	217	210	141	95.6	72.4	59.2	37.9	33.2	29.3

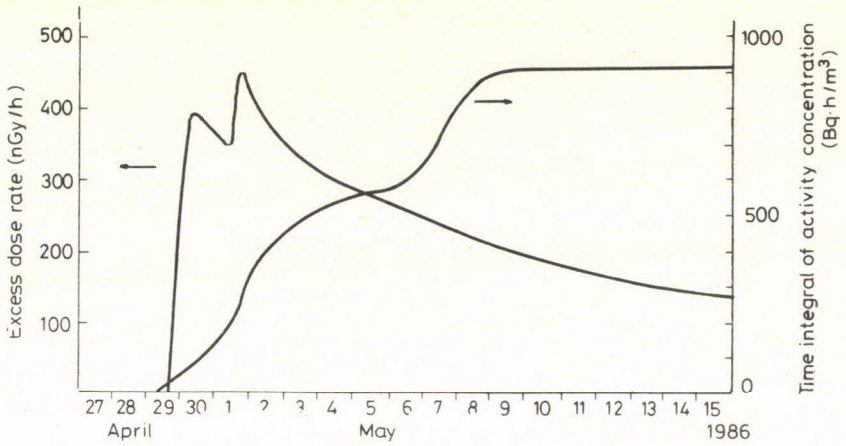

Fig. 3. Excess gamma dose rates measured by GM monitors in the CRIP and the time integral of ^{131}I activity concentration in air as a function of time (Láng, 1986)

The data, shown in Table 2, give information about the radioactive pollution level of vegetables (spinach, lettuce, sorrel, cabbage etc.) and fruits (strawberry, raspberry, apple, pear etc.) in Hungary in 1986. The samples — the No. of the samples was between 275 and 536 — were collected after the nuclear accident. The SD (standard deviation) values are rather big, this proves the inhomogeneous distribution of the contamination from the different radionuclides (Table 2).

Table 2

Radioactive contamination of the vegetables and fruits in Hungary in 1986
(Liszonyi, Kiss, 1987)

Sample	Activity Bq/kg		
	^{131}I	^{134}Cs	^{137}Cs
Fruits	35±52	17±18	31±31
Vegetables	38±185	15±29	20±53

So, although the radiation effect on the plants in Hungary was rather different in dependence of the geographic place, we must stress that the maximum radiation dose was also in the stimulation dose-range, and rather far from the inhibition one. Figure 4 indicates that the ambient radiation is insufficient for optimal supply of the essential functions (Luckey, 1980). Because stimulation was found with low doses of ionizing radiation, the ionizing radiation levels somewhat above ambient should therefore be beneficial for many physiologic functions.

The dose rate in Hungary at the beginning of May 1986 was appr. 400 nGy/h. To establish the dose burden on the plants we can calculate with ~ 100

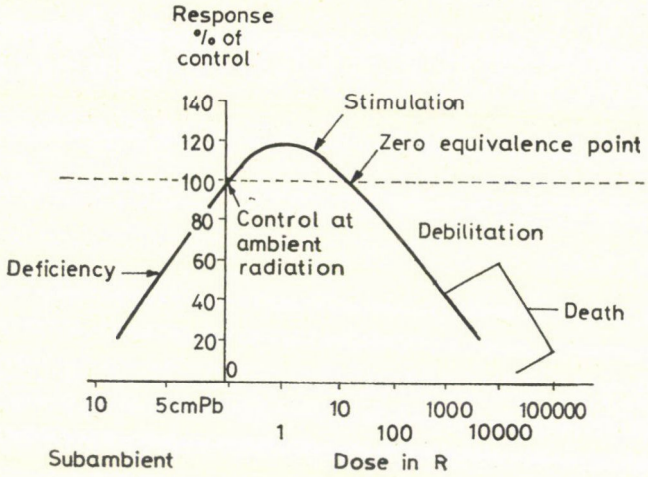

Fig. 4. **Complete dose-response curve for ionizing radiation. The zero equivalence point (ZEP) shows there is not detectable response at this dose according to the parameter being tested (Luckey, 1980)**

days vegetation period, as follows:

$$400 \times 24 \times 100 \sim 10^6 \text{ nGy}$$

This dose burden is from the external γ radiation (soil and atmosphere). Of course, to estimate the real dose burden of the plant tissues we have to take into consideration the effect of the incorporated radionuclides, and the effect of radiation, as well. In May 1986 the most important part of the radioactive contamination was originated to ^{131}I. Later — in consequence of the rather short half life ($T_{1/2}$ = 8,1 days) — the ^{131}I activity significantly decreased, and the relative portion of radiocaesium isotopes (^{134}Cs, ^{137}Cs) increased. I mention, that the plants take up the radioiodine and radiocaesium mostly through the leaves, and not indirectly through the root system from the soil. The measure of this direct uptake depends upon the radioactive pollution of the fall-out (in Hungary mainly wash-out and rain-out).

I would like to mention that the ESNA (European Society of Nuclear Methods in Agriculture) organisation has a special working group ("Radiation induced stimulation effects in living organisms") and in this working group we discuss at the annual meetings the newest information in the radiostimulation, i. e. the biopositive effect of various radiation on living organisms. It is well known that the stimulation — with optimum dose-treatment — has favourable influences on the plants (e.g. yield-increase) (Berezina (1975) Simon, Bhattacharya (1977). By the way the expression "radio-stimulation" originates from

Breslavets (1946), who meant by it the positive biological effects of radiation treatments.

I mention that in our earlier experiments with presowing radiation treatment — irradiation of the seeds before the sowing — the optimum dose was in the 1-15 Gy dose interval, in dependence of the variety of the different plant seeds (Szabó et al., 1983). Although this dose is much higher than the dose burden of the plants in Hungary from the Chernobyl accident, it is necessary to take also into account the different radiosensitivity of the plants and of the seeds. The reason is that the radiosensitivity depends mainly on the water-concentration of the material; so the optimum stimulation dose is significantly lower by the vegetative parts (water content $\sim$ 90%) than by the seeds.

To the observations, carried out in Hungary in May and June 1986, the various vegetables were growing very intensively and rapidly. This question was discussed and analysed also at the XVIIIth annual ESNA meeting in Stara Zagora, Bulgaria, 1987, by the working groups; "Radiation induced stimulation effects in living organism" and "Environmental pollution" (Szabó, 1987). Many specialists — e.g. A. Dmitriev from USSR., A. Eriksson from Sweden — reported similar observations, such as a significant increase in the green biomass of the plants, probably in consequence of the higher radiation dose level.

Of course, to carry out an exact investigation to verify this hypothesis we need a correct mathematical-statistical evaluation of the yields. The most complicated question is what to take as a control for comparison. Since the growth of the plants, the crop production and the yield depend also upon other parameters (e.g. fertilization, meteorological conditions), it is not possible to do a mathematical analysis. I mention that Simon and Bhattachariya (1977) published an interesting book about the status and future prospect of radiation stimulation, collecting data also about the fall-out stimulation experiments.

As a conclusion we can say that although the radiation effect on the plants in Hungary — in consequence of the Chernobyl accident — was rather different in dependence of the geographic place, but also the maximum radiation dose was in the stimulation dose-interval, and not in the inhibition one.

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Plant physiology and biochemistry

BIOMASS PRODUCTION OF SOME CULTIVATED AND WILD AMARANTH SPECIES

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The biomass yield and chemical composition of the different amaranth species were examined through 3 subsequent years in the great Hungarian Plain. The nutrient demand of the plants was satisfied by applying 800 m³/ha liquid manure from a specialized pig farm corresponding to 395 kg/ha nitrogen, 205 kg/ha phosphorus and 384 kg/ha potassium as active ingredients.

In spite of the dry climate and adverse soil conditions the examined amaranth species were capable of producing 15–21 t/ha dry matter when harvested at wax ripeness. The chemical composition of the plant was closely related to its age and the cutting managements. Prior to flowering, the young shoots and leaves contained 11–13% and 20–26% crude protein, respectively. The most marked decrease in crude protein content was noted from the phenophase of flowering.

Due to the quality deterioration of the older amaranth we studied the effect of early harvest on yield and quality of green forage. Investigations have shown that cutting, which removes terminal and axillary meristems, results in poor subsequent regrowth and low late-season production. The dry matter yield was significantly lower in the case of 2 outs/year (6.14 t/ha to 12.98 t/ha).

Considering grain yield, the pink grain type *Amaranthus caudatus* proved to be the most productive: 3.31 t/ha. From among the black grain varieties, *A. hypochondriacus* was found to give the highest yield and a yellow variety of the some species displayed similarly good productivity.

The protein content of amaranth was lower than in the leguminous species but higher than cereals. Compared to horse bean, more methionine, cystine, serin, glycine, valine, thyrosine and proline, but less arginine, alanine and phenylalanine were found in the amaranth grains.

Keywords: biomass production of amaranthus, *Amaranthus caudatus*, *A. hypochondriacus*, *A. cruentus*, *A. albus*, *A. retroflexus*

Introduction

The chances of domestication and renewal cultivation of the various amaranth species were investigated, in order to promote the utilization of drought-exposed areas of adverse soil characteristics of the Great Plain. The amaranthus, domesticated in Middle-America and cultivated since pre-historical times, may be subdivided into three species. In Middle- and North-West-Mexico, the *A. hypochondriacus* and the *A. cruentus* and on the southern slope of the Andes, the *A. caudatus* were cultivated. Due to favourable nutritive features, their grains were used for various purposes. When compared to cereals

which play an important part in the nutrition of humanity, amaranth grains have proved to contain much more proteins, oils and fibres, and their amino acid composition is more favourable. Due to these features as well as to its favourable nutrient utilization, the investigation of amaranths seems particularly promising from the aspect of utilizing areas previously irrigated with liquid manures.

Due to their high yield potential and favourable amino acid composition, amaranth species are being investigated world-wide with increasing interest; Ruttle (1976), Marx (1977), Leidner (1978), Lazányi et al. (1987).

The young shoots contain proteins rich in digestible essential amino acids (Martin, Roberté, 1975). Mohideen et al. (1982) in India and Makus and Davis (1984) in the United States of America obtained amaranth varieties suitable for salad. The new salad plant grows to a height of 90 to 110 cm and its leaves may be picked as early as 20 days after sowing. When harvested continually, these varieties may achieve yields of 30 to 35 t/ha.

Others, like Hauptli, Jain (1977), Anonymus (1978), recommend cultivated and wild amaranths to be utilized as green forage. These authors emphasize the advantages of their good adaptation capacity, excellent water utilization, favourable photosynthetic activity (C-4), and high yield potential. According to Lexander et al. (1970), from among 29 plant species the leaves of *A. caudatus* was the most suitable for large scale production of leaf protein concentrates, far ahead of horse beans, vetch species, herbaceous plants and alfalfa. Based on this fact, the authors concluded that protein extracts suitable for human consumption may be taken from amaranths.

Amaranths grown for their grains are no less important. The grains are at present consumed in several countries Kauffman (1982). Their popularity is due to their very good digestibility and to the short vegetation period of the species. Downton (1973) and Kauffman (1980) accentuate the importance of the amaranth in the food industry. They stated that it is the most adequate complement to foods prepared from wheat, corn and rice, since it has a high lysine, methionine and low lecithine content. Ockerman (1978) and Becker et al. (1981) investigated the chemical composition of amaranth grains. Stiebritz et al. (1985) prepared flour crepe, pastry, and poppy from amaranth grains. In the U.S.A. a Research Center sponsored by the Rodale Publishing House was established in order to collect, identify and deposit amaranth genetic resources Hass (1980), Grubben, Sloten (1981) and Kauffman, Reider (1984).

Research workers of other nations also entered this activity and contributed valuable information to our common knowledge on the extent of self-fertilization in amaranth species and its importance for systematization; Jain et al (1982). The characteristics and the heredity of the starch in the grains were investigated by Okuno, Sakaguchi (1981, 1982).

Materials and methods

Field trials were performed in the Research Institute of the Debrecen Agricultural University on a meadow solonetz soil with a deep humus layer, developed on a calcerous loess. The humus content of the cultivated soil layer amounted to 2.86%, the pH value of the same to 6.2. Its C_A number was 50. According to our analysis, the soil was well supplied with potassium, fairly with nitrogen and poorly with phosphorus.

The soil of the farm scale trials was meadow solonetz, in a state of slow transformation to steppe. It was irrigated during the growing period with 800 m^3/ha liquid manure, corresponding to 395 kg/ha nitrogen, 205 kg/ha phosphorus and 384 kg/ha potassium as active ingredients. The trial series was continued from 1985 to 1987. Weather conditions were in all three years characterized by drought, being particularly severe in 1986.

In our trials, sowing was performed from 15th to 25th April. The small plot trials (10 m^2/parcelle) were arranged at random, and the farm scale experiment on plots of 0.2 to 0.5 ha. The seeding rate was from 300 to 500 g/ha; this amount provided a sufficient number of plants. Amaranth grown for green fodder was sown at a row distance of 20 cm; that grown for grains, at one of 60 cm. in the small plot trials, the total biomass yield was assessed even in the latter case.

Amaranths grown for green fodder were harvested prior to the appearance of the inflorescence, while the types grown for grain were harvested — in order to reduce losses — at wax ripeness, and threshed following a post-ripening period of 1 to 2 weeks.

The chemical analyses presented in this paper were performed at the Laboratory of the Research Institute, and amino acid contents were determined at the Agricultural University.

Results

Agronomical evaluation of amaranth grown for green fodder

Wild amaranth has traditionally been an excellent quality green fodder plant for pigs. Consequently, the biomass yield and dry matter content of the different amaranth species were examined through 3 subsequent years in the dry areas of the Great Plain.

In spite of the fact that the 3 years under investigation were drier than average, the species examined were capable of producing 15 to 21 t/ha of dry matter when harvested after flowering, at wax ripeness (Table 1). The maximum of green yield during the 3 years was harvested from each species in 1985 (55,8 t/ha). The species *A. hypochondriacus* and *A. Albus* proved to be most productive. *A retroflexus* and *A. blitoides* yielded in the first year less than the other species; therefore their investigation was discontinued.

The fodder value of the amaranth grown for green forage is primarily determined by the specific features of the species, by the climatic and sol conditions at the growing site, and by the time of harvest. In the course of ripening the dry matter content in the plants increases but from the phenophase of flowering protein content, nutrient concentration and digestibility decrease.

Table 2 shows the changes in protein, phosphorus and potassium content of the cultivated amaranth species. Prior to the appearance of the inflorescence, 11 to 13% crude proteins are present in the stem of the young shoots and 20 to 26% in the leaves. During the phenophase of flowering crude protein content

Table 1

Green and dry matter yields of the cultivated and wild amaranth species investigated at wax ripening

Species	Green yield t/ha				Dry matter yield t/ha			
	1985	1986	1987	Average	1985	1986	1987	Average
A. caudatus (P)	48.9	43.5	44.3	45.6	13.6	15.3	20.4	16.4
A. hypochondriacus (B)	62.1	51.7	56.5	56.8	18.1	19.6	26.8	21.5
A. cruentus (B)	56.3	30.3	48.1	44.9	16.1	12.8	16.5	15.1
A. albus (B)	61.9	49.9	52.2	54.7	17.8	15.5	25.9	19.7
A. retroflexus (B)	54.3	—	—	—	14.3	—	—	—
A. blitoides (B)	26.5	—	—	—	7.0	—	—	—
A. caudatus (Y)	51.0	46.7	43.5	47.1	14.4	16.6	16.9	16.0
A. hypochondriacus (Y)	56.2	73.2	53.3	60.9	14.3	21.8	23.9	20.0
Averages	52.2	49.2	49.7	51.7	13.7	16.9	21.7	18.1
LSD 5%	4.08	3.14	4.64	—	2.10	1.68	1.69	—

Grain colour: P = pink, B = black, Y = yellow

decreased in the stem to 4 to 5%, and in the leaves to 14 to 15%, while spikes had a protein content of 18 to 21%. In wax ripening, protein content in the leaves continued to decrease, amounting only to 12 to 15%; and the protein content values in the spikes similarly decreased.

Table 2

Changes in the chemical composition of the cultivated amaranth species during the vegetation period (D. S. %)

Species	Prior to flowering		During flowering			During wax ripening		
	stems	leaves	stems	leaves	inflo-rescen	stems	leaves	inflo-rescen
A. caudatus (P)								
Crude protein	15.63	26.98	5.17	18.25	23.16	4.76	14.52	20.37
P_2O_5	1.65	1.58	0.87	0.76	1.10	0.58	0.82	0.97
K_2O	9.40	5.80	4.37	5.84	5.17	5.43	4.46	4.63
A. hypochondriacus (B)								
Crude protein	11.38	20.88	4.63	11.48	21.13	4.38	12.38	19.31
P_2O_5	1.48	1.24	0.76	0.78	0.97	0.62	0.78	0.82
K_2O	8.65	5.80	5.75	5.25	4.40	6.00	4.40	4.42
A. cruentus (B)								
Crude protein	12.75	26.38	4.75	15.38	18.13	4.50	15.00	13.25
P_2O_5	1.23	1.33	0.40	0.74	1.18	0.40	0.55	1.00
K_2O	8.65	4.85	3.25	6.00	4.85	4.00	5.00	4.85

Grain colour: P = pink, B = black

Table 3

Green and dry matter yield of amaranths grown for green fodder, in two cuts yearly

Species	Green yield kg/21.2 sqm			t/ha	Dry matter yield kg/21.2 sqm			t/ha
	1st cut	2nd cut	Total		1st cut	2nd cut	Total	
A. caudatus (P)	41.70	25.00	66.70	31.46	6.06	11.87	17.93	8.46
A. hypochondriacus (B)	46.20	6.50	52.70	24.86	8.18	4.84	13.02	6.14
A. cruentus (B)	55.70	7.00	62.70	29.57	10.13	4.22	14.35	6.77
A. albus (B)	60.50	14.00	74.50	35.14	9.60	10.04	19.64	9.26
A. caudatus (B)	50.90	28.50	79.40	37.45	8.35	13.68	22.03	10.39
A. hypochondriacus (Y)	52.70	51.10	103.80	48.96	8.09	19.42	27.51	12.98
A. retroflexus (Y)	43.30	40.30	83.60	39.43	7.05	17.13	24.18	11.41

Note: first cut prior to flowering, second cut at the end of the vegetation period. Grain colour: P = pink, B = black, Y = yellow

In 1986, due to the quality deterioration in the older amaranth stems and leaves of plants grown for fodder, we investigated the possibility of cutting the stand several times. The results are presented in Table 3. It may be stated that the dry matter yields obtained in two cuts per year were lower than those obtained when harvest followed the opening of blossoms. The majority of the species yielded less than 10 t/ha. The highest green mass yields and, at the same time, the highest dry matter ones were obtained with the yellow grain variety of *A. hypochondriacus*. This fact may be explained by the longer vegetation period and by the intense branching capacity. Due to its good sprouting, *A. retroflexus* also offered high yields. At the same time protein content and digestibility also decreased.

The amino acid composition in the young shoots of amaranth species is presented in Table 4 where the same values for young alfalfa shoots are shown for control.

According to our investigations, the protein content in amaranth species is lower than in the young alfalfa shoots. From among the amino acids, larger quantities of methionine, alanine, phenylalanine and glutamin acid, were found while proline, hystidine and asparagine acid contents were lower. Great differences were observed in the lysine content of the young shoots of the investigated amaranth species. The lysine content of the yellow grain *A. caudatus* and *A. hypochondriacus* were considerably higher than those of alfalfa.

Assesment of the grain productivity of amaranth species

Like the cereals, amaranths contain starch, and were grown for their grain yield. Due to this fact, in our small plot and farm scale trials the grain

Table 4

Protein content (%) and amino acid composition

Species	Protein (%)	ASP	THR	SER	GLU	GLY	ALA	CYS
Young shoots								
A. caudatus (P)	25.3	12.41	5.00	6.75	14.57	4.90	8.89	0.44
A. hypochondriacus (B)	27.4	12.06	6.13	5.94	15.38	4.96	8.96	0.22
A. cruentus (B)	27.3	11.07	6.34	5.32	15.18	4.78	8.62	0.38
A. albus (B)	26.9	12.14	6.92	5.32	15.62	5.20	8.26	0.47
A. retroflexus (B)	24.2	12.60	4.74	5.29	12.25	4.92	9.10	0.40
A. blitoides (B)	20.6	11.91	6.62	5.18	14.47	4.67	7.94	0.17
A. caudatus (Y)	24.7	6.22	2.57	2.69	10.15	6.05	3.78	0.87
A. hypochondriacus (Y)	25.7	8.98	3.71	4.60	14.05	6.29	4.82	0.70
Medicago sativa	29.2	16.37	4.18	5.48	10.24	5.40	4.83	0.38
Grain								
A. caudatus (P)	16.3	7.82	2.96	5.79	17.56	6.06	3.10	1.20
A. hypochondriacus (B)	16.3	6.22	4.19	5.89	14.22	7.04	2.66	0.60
A. retroflexus (B)	14.8	6.83	4.35	6.41	14.00	7.13	2.97	0.70
A. hypochondriacus (Y)	16.5	7.26	3.32	4.50	15.82	6.82	3.33	0.60
Vicia faba	31.8	7.94	3.30	4.03	15.62	3.26	2.82	0.34
Pisum sativum	19.8	9.96	3.13	3.65	16.00	3.79	4.44	0.19

Grain colour: P = pink, B = black, Y = yellow

productivity of the available cultivated and wild species was investigated in the unfavourable soil conditions prevailing in some areas of the Great Plain. The production values obtained during our trials are shown in Table 5.

Table 5

Grain yield of the major cultivated and wild amaranth species under dry conditions prevailing in the Great Plain

Species	Grain colour	Grain yield t/ha					Thousand grain-weight
		1985	1986/a	1986/b	1987	Average	
A. caudatus (P)	pink	3.63	3.05	3.40	3.14	3.31	0.7
A. hypochondriacus (B)	black	3.26	2.27	3.23	3.62	3.10	0.8
A. cruentus (B)	black	3.20	1.87	2.97	2.77	2.70	0.8
A. albus (B)	black	2.54	2.08	2.32	3.55	2.62	0.7
A. retroflexus (B)	black	2.41	—	—	—	—	0.4
A. blitoides (B)	black	1.26	—	—	—	—	0.8
A. caudatus (Y)	yellow	2.16	1.76	1.83	2.47	2.06	0.8
A. hypochondriacus (Y)	yellow	2.55	1.69	1.53	3.32	2.27	0.8
Averages:		2.63	2.12	2.54	3.15	2.68	
LSD 5%		0.431	0.153	0.265	0.273	—	

(g/100 g protein) *of amaranth species*

VAL	MET	ILE	LEU	TYR	PHE	LYS	HYS	ARG	PRO
6.30	1.32	6.19	6.46	2.36	4.53	4.22	1.57	4.48	1.63
6.46	1.28	4.56	5.97	2.43	3.95	4.49	1.77	4.50	2.78
6.24	1.75	5.24	6.82	2.38	4.44	4.00	1.57	4.07	2.77
5.42	1.07	6.03	6.68	1.87	4.60	4.28	1.74	4.18	1.72
6.31	1.10	4.96	7.06	2.00	5.19	4.78	2.22	6.59	2.57
7.07	1.04	4.94	6.86	2.55	4.90	5.04	2.46	4.36	1.89
4.69	1.11	4.19	8.01	2.53	5.46	9.33	1.85	9.50	3.18
6.55	1.17	4.75	7.45	2.68	3.83	8.93	2.11	4.03	7.36
6.39	0.59	5.12	7.25	2.41	2.28	5.91	3.00	5.51	5.73
5.19	1.57	3.47	5.23	2.60	4.51	6.51	3.77	7.90	5.65
5.43	3.31	3.28	4.83	3.20	4.07	6.78	3.59	7.61	9.62
4.94	2.06	3.56	5.20	3.80	4.32	6.69	3.71	7.47	8.38
5.07	1.87	3.50	5.21	3.42	4.44	7.51	3.80	8.98	7.21
4.73	0.63	3.62	7.29	3.17	3.75	6.77	3.51	8.28	13.56
3.88	0.61	3.82	6.17	2.94	5.27	6.51	2.78	15.96	2.77

The pink grain variety of *A. caudatus*, proved to be most productive. Even the yield fluctuations were the lowest in this species. From among the black grain varieties *A. hypochondriacus* was found to yield the highest, but the cream-coloured variety of the same species displayed a similarly good productivity. The thousand-grain-weight values for amaranths are below 1 g. The lowest thousand-grain-weights were found in the wild *A. retroflexus*.

The protein content found in the cultivated amaranth species was lower than in the leguminous species (Table 4). With respect to the essential amino acids, their amino acid composition is more favourable than in the latter. Compared to horse beans, more methionine, cystine, serine, glycine, valine, thyrosine and proline, but less arginine, alanine, phenylalanine and asparagine acid were found in the amaranth grains.

Discussion

In the dry areas, tending to drought with unfavourable soil conditions of the Great Plain, the biomass production and the grain yields of various cultivated and wild amaranth species were investigated. In growing amaranths

we attempted to meet the needs of plants to an optimum level, in order to study the potential productivity of the species. Farm scale trials were performed in fields irrigated with liquid manure in order to provide nutrient supply at low costs and at a high level. In our investigations we stated that, on the average of several years, maximum green mass and dry matter yields were obtained with *A. hypochondriacus*. Concerning green mass yield, a yellow grain variety (60.9 t/ha) and, concerning dry matter yield, a black grain variety (21.5 t/ha) proved the best when the stock was harvested once a year at wax ripening.

When harvested prior to opening of the flowers, the green and dry matter yields of amaranths are lower but the quality of fodder is higher. The protein content of the plants approximates that of alfalfa, while its amino acid composition proved more favourable in several aspects than alfalfa. Primarily methionine, alanine, phenylalanine and glutamine acids are present in larger quantities.

Was the grain productivity of the cultivated and wild amaranths is assessed, it may be stated that in a pink grain variety of *A. caudatus*, average grain yields of 3.31 t/ha were obtained. When compared to the protein content of the cereals like wheat, corn, and rice, all playing an important role in human nutrition, those of amaranth grains are almost twice as high. Their amino acid content is also favourable and, due to their high lysine and methionine content, they may be considered important complements to cereals.

The growing of amaranths in areas irrigated with liquid manure is recommended where high nitrogen demands of this plant may be met at low cost, or in the neighbourhood of specialized animal farms, where the green fodder obtained may be used on the spot or processed, which reduces feeding costs quite reasonably. The growing of amaranths offers a new and economic method of utilizing areas irrigated with liquid manure, and simultaneously contributes to the solution of environment pollution problems.

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FROST TOLERANCE AND PRODUCTION OF *SALVIA SCLAREA L.*

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At the Research Institute for Medicinal Plants in 1985—1987, a *Salvia sclarea* population was studied for overwintering, yield and active substance, and flowering biology characteristics. In the first year 15% of the population, while in the following year 100% of the overwintered plants, flowered. According to the statistical analyses, both the first year flowering and the difference between cropyears are important from the point of view of frost damage. There was a correlation between first year flowering and frost damage in each year. Of the parameters of flowering biology the number of flower stalks per plant and the stalk height are lowest in plants beginning to flower in the first year. These plants develop in the second year shorter inflorescences than the other plants. The primary branches were fewer in the second productive year in each plant. The totalled fresh and essential oil yields of 2 productive years were considerably larger for plants beginning to flower in the second year, while in sclareol output no difference was found between the two types of plant.

Keywords: essential oil output, frost tolerance, *Salvia sclarea*, sclareol output

Introduction

The *Salvia sclarea* L. is an important essential oil plant indigenous to the Mediterranean areas of Europe and cultivated for several decades in Hungary Its essential oil is utilized in the perfume- and aroma industry (Hornok, 1978, Rácz et al. 1984). For essential oil extraction, the inflorescences are gathered in at the full waxen ripeness of seed, and are freshly distilled. The components of the essential oil are esters and alcohols, the main component is linalyl acetate. In these days much interest is shown in the sclareol, a diterpene alcohol to be found in the plant, which in perfume composition is the initial material for the synthesis of amber-scented substances (Gildmeister 1961, Guenther 1948, Sváb 1978, Kernóczi et al. 1985, Lawrence 1979). It is produced from the plant material left behind after the essential oil distillation.

The *Salvia sclarea* is a rosette plant, which usually flowers in the second cycle of vegetation. In its natural area it is a perennial (Soó 1968), but even in a colder climate it may survive for several years (Guenther 1948, Gulati 1980, Szavcsuk 1975). As a result of breeding work, annual varieties and such perennials that produce flowers in the first year have been developed (Arinstejn

1975, Ilieva 1979, Vlaszova 1986). In the cultivation of these varieties increased importance is attached to winterhardiness. With the *Salvia sclarea* — as experiences show — the yields of the second- and third-year stands are influenced by the weather conditions of the vegetation period and the percentage of overwintering (Arinstejn 1975, Szavcsuk 1975). Arinstejn et al. (1975) noticed that in Moldavia the difference between the annual and biennial forms was not constant but depended on the year. They found a close negative correlation between the number of plants flowering in the first year and the number of those surviving the winter. According to Szavcsuk (1975) yield can only be expected in the first year when in the period between emergence and rosette development the mean temperature is 10-15 °C. A close correlation was found between yield and precipitation, and a medium one between yield and temperature. On the average of eight years a 24% destruction by frost was registered for the variety examined.

At the Institute investigations were started to find out how in a *Salvia sclarea* stand the plant number per unit area and the plant material production would change in the course of three years.

The examinations here described form the basis and first phase of an experiment series the purpose of which is to acquire a more thorough knowledge of the production biology of *Salvia sclarea* plants.

Materials and methods

On the experimental area of the Institute at Budakalász a half-hectare stand of *Salvia sclarea* was examined over a period of three years. In March 1985 seed of the strain 31 from the selection of the Institute was sown in rows spaced at 70 cm. The soil was a medium compacty (KA = 44) alluvium with a medium humus content (2.9%). The trend of temperature, the heat sum and the temperature conditions of the examination period are shown in Table 1 and Fig. 1. The stand was subjected to the usual agrotechnical operations. In the first year experimental plots of 2 m^2 (3m) each were laid out in 25 replications. On their areas the total plant number, the number of flowering plants, some data of flowering biology (stalk/plant, stalk height, length of inflorescence, number of branches) and the values of components and yield were recorded every year.

In the first year the following plants were marked, and in the subsequent year their characteristics were separately recorded. Harvesting was always carried out in the phase of development when the seeds in the lower branches began to turn brown.

The data were statistically evaluated, then independence and significance examination as well as main component analysis were made after Sváb (1979, 1981).

Results and evaluation

In the first year the data of 21 plants were recorded for each experimental plot on an average, of which an average of 3 plants — 15% — flowered in the year of sowing. Similar data are published in the relevant literature (Vlaszova, 1986). The other plants remained in rosette stage. The first winter (1985–86) was survived by an average 14 plants, that is, the original plant

Table 1

Meteorological data characteristic of the period of examination from 1 November to 31 March in 1985/86 and 1986/87)

Heat sum (°C)	276	91
Number of frosty days (min. 0 °C)	69	30
of which days without snow-cover	20	91
Number of winter days (max 0 °C)	40	64
of which days without snow-cover	7	20
Number of rigorous days (min. 10 °C)	10	19
Total amount of precipitation (mm)	300	227
of which snow (mm)	104	114

Month	Mean temperature (°C)		
	1985/86	1986/87	50-year's average
January	−0.5	−3.2	−0.8
February	−2.4	0.7	1.1
March	3.3	0.9	6.2
November	3.5	5.3	5.3
December	3.8	−0.5	1.3

number was reduced to 66%. With this average analysed in detail it was found that the plants flowering in the very first year perished by frost in a significantly larger proportion than those remaining then in a rosette stage (Table 2, Fig. 2). This in essentials agrees with the observations by Arinstejn et al.

In the second year all overwintered plants developed flowers. By the third year the total original number of plants was reduced by two-third, to 7 plants per plot on an average, that is, a further 57% perished by frost compared to the second year. By the third year, of the plants beginning to flower in the first year, 94% while of those flowering from the second year only 61% perished in frost.

Table 2

Percentage of plants perished by frost in the two types of plant and in the winter periods examined

	After the*		Average
	1st winter	2nd winter	
A type	64.68	94.40	79.54
B type	29.28	45.96	37.62
Average	46.98	70.18	7.40
$LSD_{5\%}$			

* compared to the previous year

Winter-injury is supposed to be greatly influenced by three factors: the weather, the age of the plant and the first-year flowering. As to the importance of these factors we established the following: according to the statistical correlation examination first-year flowering and winter-injury were in correlation both in the first and in the second winter of the experiment. The correlation was closer in the first year (Table 2).

According to a bifactorial variance analysis both factors — i.e. first-year flowering and cropyear — have a significant influence on winter-injury. Of the plants flowering in the first year a larger proportion perished by frost than of those remaining then in a rosette stage (Table 2). Out of the two winters of the experiment, the 1986–87 winter gave a higher proportion of winter-injury. This was true both in the case of plants flowering in the first year and with those developing flowers first in the second year, and as regards the total plant number alike. With a knowledge of the meteorological data this can be traced back also to the much larger number and less favourable distribution of frosty days in the second than in the first winter (Fig. 1, Table 1).

From the above we can draw the conclusion that first-year flowering has a negative effect on overwintering, and beyond this, the weather conditions of a given winter also influence the winter-injury.

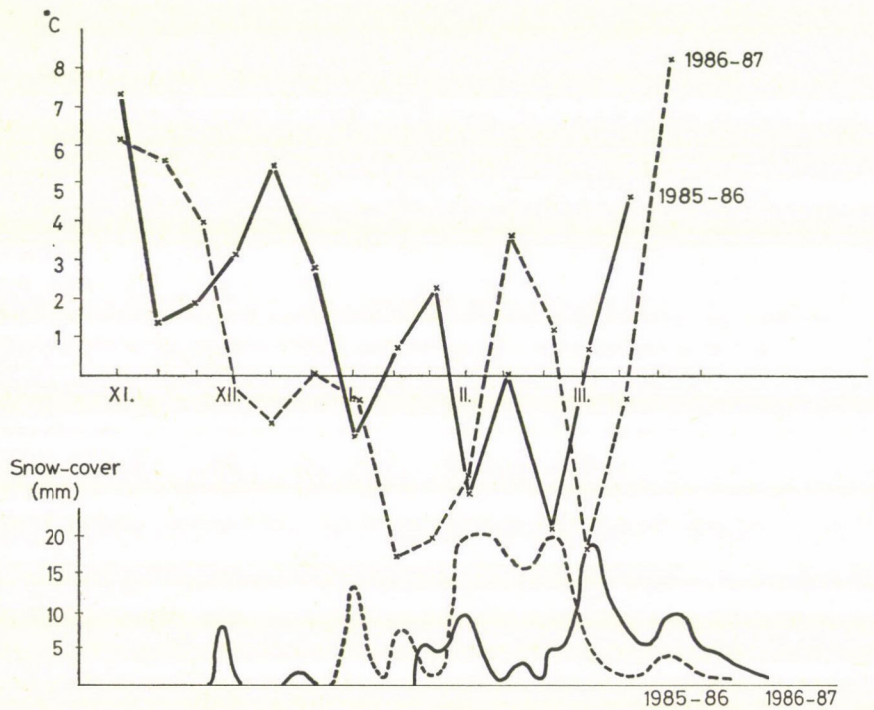

Fig. 1. Trend of temperature and thickness of snow-cover

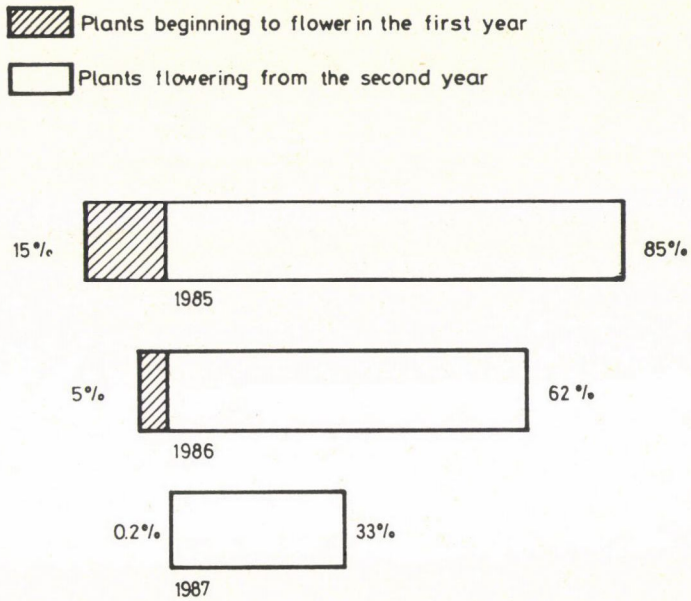

Fig. 2. Decrease in the plant density of the stand examined due to frost-injury (in percentage to the original plant number)

With plants beginning to flower in the first year the stalks are fewer and the average stalk height is smaller than with those flowering from the second year (Table 3). Vlaszova (1986) published similar data.

As for the length of inflorescence there is no significant difference either between the two types of plant, or between the two years of flowering, on an average.

Table 3

Morphological features and yield valueq of the plant groups examined

	Stalk/plant n	Stalk height cm	Length of inflores. cm	Branch/stalk n	Essential oil % dry matter	Sclareol % dry matter	Fresh yield g/plant	Essential oil yield mg/plant	Sclareol mg/plant
1985. Plants flowering first *A*	2.6	60.0	39.6	5.9	0.41	0.97	73.4	105.3	213.7
1986. Plants flowering the second time *A*	2.4	68.6	23.4	3.8	0.26	0.90	22.3	78.3	80.9
1986. Plants flowering first *B*	4.2	70.1	36.9	6.3	0.94	1.25	52.6	98.9	129.5
1987. Plants flowering the second time *B*	3.9	72.8	37.5	3.9	0.78	0.99	74.7	143.5	181.6
$LSD_{5\%}$	1.7	8.75	12.13	1.77	0.34	0.27	27.2	20.68	128.8

Within the inflorescence the number of primary branchece is lower on the second than on the first occasion of flowering, whether it is a plant flowering from the first year or one flowering from the second year; it is thus an ontogenic effect.

In the fresh yield significant difference was only observed on the second-year flowering of plants beginning to flower in the first year; then the yield was significantly lower than in the other cases. In the second productive year (3rd year of life) of plants flowering from the second year such an extent of fresh yield reduction was not experienced (Fig. 3, Table 3).

There is a significant difference in essential oil content too between the two types of plant, but that it was the first or the second productive year of the plant was not of much importance. The lower essential oil contents of plants turning into flowering earlier are referred to by literary data, too (Ilieva and Peneva 1983, Vlaszova 1986, Zámbó 1984), though it may be a year effect as well (e.g. lower number of sunshine hours). The largest quantity of essential oil was obtained in the first and third year of cultivation, due first of all to the volume of fresh yield (Table 3). An examination of the joint essential oil outputs of 2 productive years reveals that the essential oil content is significantly higher in plants flowering from the second year of cultivation (Fig. 3, Table 4). The sclareol content of the plants, on the other hand, did not vary either with the type of plant or with the period of flowering.

Table 4

Joint values of yield for the two types of plant on the basis of two productive years

	Fresh yield g/plant	Essential oil yield	Sclareol yield
		mg/plant	
A-type plants	95.70	183.60	294.60
B-type plants	127.30	242.40	311.10
$LSD_{5\%}$	35.37	55.98	64.51

The plants flowering from the first year produced much less sclareol than in the other flowering periods. In the totalled sclareol outputs of 2 productive years, there was no difference between the two types of plant (Fig. 3).

To decide whether it was the type of flowering or the difference between cropyears that had the greater effect we carried out a main component analysis. The correlations between the factors show that the frost-injury is in close negative correlation with both the number of stalks per plant and the length of inflorescence as well as with the essential oil- and sclareol content. Close

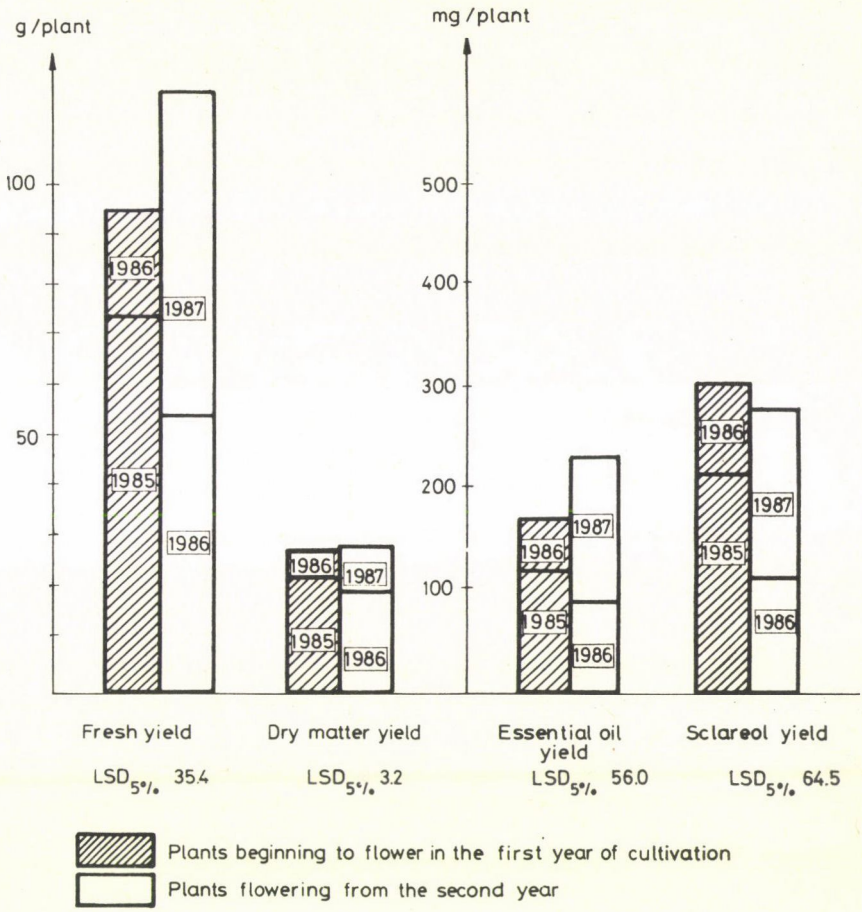

Fig. 3. Production by the two types of plant in the years of the experiment

positive correlation was found between the essential oil content, the stalk number per plant and the sclareol content (Table 5).

When plotting the observation units we found considerable differences both between the two types of plant and between the years of cultivation. Within this the two types of plant show greater differences in frost sensitivity, essential oil- and sclareol content, while as regards the stalk height and number of branches — in short: the characteristics of flowering — the difference between the successive cropyears is dominant.

It can be established that according to the parameters considered the differences between plants starting to flower in the first year of cultivation and those flowering from the second year are greater than the differences between the successive productive years.

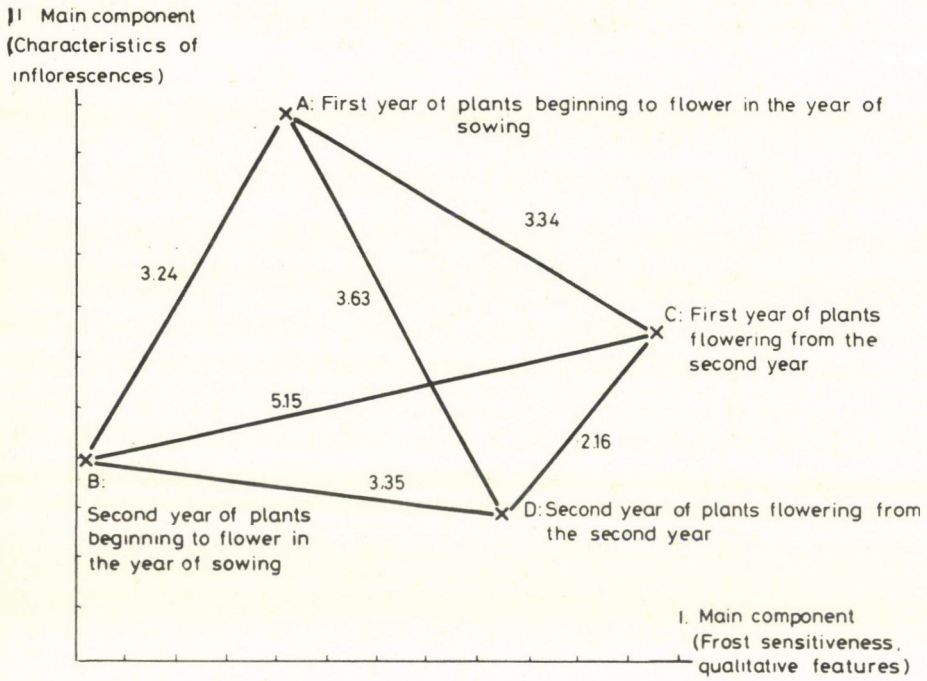

Fig 4. **Configuration of the observation units on the basis of main component analysis**

Table 5

Outstanding values of the correlation matrix of the main component analysis
($r > 0.7$)

	1.	2.	3.	4.	5.	6.	7.
Frost-injury	1	−0.928*	−0.801*	−0.967	−0.323	−0.563	−0.855*
Stalk/plant	−0.928*	1	0.566	+0.991*	0.652	0.268	0.797
Length of inflorescence	−0.801	0.566	1	0.639	−0.171	0.596	0.485
Essential oil content	−0.967	0.991	0.639	1	0.551	0.387	+0.847*
Stalk height	−0.323	0.652	−0.171	0.551	1	−0.483	0.263
Branch/stalk	−0.563	0.268	0.596	0.387	−0.483	1	0.716
Sclareol content	−0.855	0.797	0.485	0.847	0.263	0.716	1

*LSD = 5%

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PHYSIOLOGICAL ANALYSIS OF NITROGEN RESPONSE IN RAPE AND TURNIP

I. LEAF AREA, DRY MATTER AND GROWTH ATTRIBUTES

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The effect of four levels of nitrogen (*N*) on leaf area, dry matter production and growth attributes of rape and turnip was studied. The higher levels of *N* reduced and delayed seedling emergence. As a result up to 25 days after sowing, high nitrogen-treated plants had lower leaf area and total dry weight. After that period leaf area and dry weight were increased significantly by increasing the *N* level. Relative growth rate (*RGR*), net assimilation rate (*NAR*) and relative leaf growth rate (*RLGR*) were markedly affected by *N*, but the effect of *N* was not marked on leaf area ratio (*LAR*), leaf wight ratio (*LWR*) and specific leaf area (*SLA*).

Keywords: dry matter, leaf area, growth attributes, rape, relative growth rate, turnip

Introduction

Although many workers both in the field Lambert 1962; Auda et al. 1966; Buttery 1969a, b; Khalifa 1973) and in controlled environments (Schmidt and Blaser 1967; Ryle 1970; Osman et al. 1977; Robson and Parsons 1978; Robson and Deacon 1978; Thomas et al. 1978) have investigated the large increase in yield which nitrogen (*N*) application can bring about, the physiological basis of this effect remains ill-defined. This may be the result of increased foliage mass at the expense of roots (Watson 1956) or it arises from increased photosynthetic efficiency (Ryle and Hesketh 1969). In wheat, Osman et al. (1977) reported that *N* variations caused differences in dry matter yield, leaf area, *RGR* and *NAR*. Similar results were obtained by Robson and Parsons (1978) in ryegrass. The present experiment was designed to study the effect of different levels of *N* on leaf area, dry matter and growth attributes. A subsequent paper will deal with photosynthesis, respiration and stomatal and leaf anatomical characters.

Materials and methods

Seeds of forage rape (*Brassica napus* cv. Lair) and turnip (*B. capestris* cv. Labra) were sown in 12.5 cm plastic pots in a glasshouse of the Welsh Plant Breeding Station, Aberystwyth, U. K. The soil composition was equal parts of unsterilized soil, grit, sand and peat. Four levels

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of N were used as follows:

N_0 = No nitrogen
N_1 = 0.4 g $NaNO_3$ per pot (37.5 kg N/ha)
N_2 = 1.6 g $NaNO_3$ per pot (150 kg N/ha)
N_3 = 6.2 g $NaNO_3$ per pot (600 kg N/ha)

In each pot 8 g "double season PK" (150 kg/ha of P_2P_5 and K_2O) were applied. The fertilizers were thoroughly mixed with the soil before sowing. The experiment was arranged as a randomized complete block design with four replications.

For growth analysis six destructive harvests were taken, with the first harvest 18 days after sowing and the subsequent harvests at weekly intervals. At each harvest two plants per treatment per cultivar were sampled from each replication. Plants were separated into leaf lamina, petiole, stem and root which were weighed after oven-drying at 95 °C for 48 h. Total leaf area per plant was measured using an automatic leaf area meter. From leaf area and dry weight, various growth attributes were calculated by the classical approach (Radford 1967). Data were analysed statistically using the analysis of variance.

Results and discussion

Mean squares from analysis of variance for leaf area, total dry weight and growth attributes are shown in Table 1. Harvest time was highly significant in all the characters and *N* level was also highly significant in all except

Table 1

Mean squares from analysis of variance for various characters

Item	df	Leaf area per plant	Total dry weight per plant	*RGR*	*NAR*
Replication	3	0.04	29.6*	1.7	4.7
Harvest (H)	5	88.40***	1711.0***	567.7***	225.9***
Nitrogen (N)	3	86.24***	1012.0***	38.9***	32.7**
Cultivar (C)	1	0.17	15.8	9.3	0.1
H × N	15	14.06***	201.6***	8.7*	9.0
H × C	5	0.41**	28.6*	14.6**	13.3
N × C	3	0.91***	4.1	0.3	1.8
H × N × C	15	0.20	6.4	13.3**	7.4
Error	141	0.12	10.9	3.3	5.9

Item	df	*LAR*	*RLGR*	*LWR*	*SLA*
Replication	3	3.38*	0.13	0.05	2.68
Harvest (H)	5	62.95***	718.11***	39.00***	206.10***
Nitrogen (N)	3	3.57*	45.43***	3.59***	5.39
Cultivar (C)	1	3.17	2.57	2.52**	2.76
H × N	15	0.48	14.51***	1.24***	3.61
H × C	5	2.58	17.89***	2.64***	3.49
N × C	3	1.08	1.40	0.54	4.76
H × N × C	15	1.03	9.05***	0.86***	4.34
Error	141	1.08	0.97	0.28	3.14

*, ** and *** indicate significant at 5%, 1% and 0.1% level, respectively.

SLA. Cultivar difference was significant in *LWR* only. The significant harvest *X* nitrogen interaction indicated that the *N* effect was different at different harvest times for leaf area, dry weight, *RGR*, *RLGR* and *LWR*. Harvest *X* cultivar interaction was also significant in those five characters, demonstrating that the two cultivars behaved differently at the different harvests. Nitrogen *X* cultivar interaction was significant for leaf area per plant.

The effects of *N* on the above mentioned characters at different stages on growth are given in Tables 2 and 3. The higher levels of *N* reduced and delayed

Table 2

Leaf area and dry weight of Lair (upper value) and Labra (lower value) with different levels of nitrogen at six harvests

N level	Days after sowing					
	18	25	32	39	46	53
	Total leaf area per plant (cm^2)					
N_0	6.1	59.9	114.1	129.4	132.4	113.9
	7.2	56.7	128.3	127.8	141.2	109.5
N_1	7.7	82.0	159.4	223.8	248.9	118.6
	5.9	98.7	178.3	246.4	237.3	171.8
N_2	5.7	79.2	282.3	432.9	596.7	620.8
	4.9	98.3	323.9	376.7	487.6	545.2
N_3	5.7	22.3	246.9	469.8	676.2	712.1
	3.0	66.8	335.3	509.3	682.3	745.5
	Total dry weight per plant (g)					
N_0	0.05	0.43	1.28	1.44	2.04	1.90
	0.05	0.33	1.10	1.41	2.70	2.27
N_1	0.06	0.47	1.75	2.58	2.73	2.44
	0.04	0.51	1.61	2.32	3.40	2.73
N_2	0.04	0.44	2.55	4.64	6.24	7.47
	0.04	0.47	2.70	3.73	6.52	8.22
N_3	0.03	0.12	2.04	5.98	7.29	8.81
	0.02	α.41	2.72	4.13	8.94	10.78

the emergence of seedlings. As a result, up to the second harvest (25 days after sowing) those plants which received N_3 treatment were smaller. After third harvest, the leaf area and dry weight were increased significantly by increasing the *N* level. Due to the senescence of some leaves of the plants which received N_0 and N_1 treatments, the leaf area and dry weight fell towards the end of the experimental period.

Osman et al. (1977) reported that *N* application increased dry weight and leaf area of wheat at the 23rd day from sowing; *RGR* and *NAR* increased from the 30th day and *LAR* from 37th day. Since *RGR* and *NAR* declined on or after the 44th day, they concluded that effects of *N* on photosynthesis, as

Table 3

Growth attributes of Lair (upper value) and Labra (lower value) with different levels of nitrogen at five harvest intervals

	Days after sowing					Days after sowing					Days after sowing					
	18–25	25–32	32–39	39–46	46–53	18–25	25–32	32–39	39–46	46–53	18	25	32	39	46	53
	RGR ($g \cdot g^{-1}\ day^{-1}$)					*NAR* ($gcm^{-2}\ day^{-1}) \times 10^{-4}$					*LWR* ($g \cdot g^{-1}$)					
N_0	0.310	0.158	0.018	0.050	−0.021	22.5	14.4	2.2	6.7	−9.8	0.390	0.592	0.569	0.482	0.478	0.3^{92}
	0.240	0.205	0.033	0.087	0.024	16.1	12.7	3.6	13.4	−4.5	0.465	0.616	0.658	0.563	0.394	0.3^{24}
N_1	0.293	0.184	0.063	−0.002	−0.010	18.5	15.9	6.6	0.8	−1.5	0.423	0.628	0.600	0.533	0.427	0.401
	0.356	0.167	0.054	0.045	−0.022	20.7	11.6	4.9	1.5	−5.8	0.427	0.711	0.675	0.638	0.459	0.422
N_2	0.337	0.251	0.088	0.041	0.028	20.7	18.9	8.7	5.0	3.0	0.380	0.649	0.624	0.560	0.547	0.505
	0.360	0.262	0.055	0.076	0.040	19.6	17.0	4.0	9.0	5.1	0.434	0.707	0.694	0.623	0.429	0.415
N_3	0.201	0.406	0.153	0.030	0.024	11.1	29.5	15.8	3.2	2.9	0.401	0.612	0.676	0.549	0.573	0.481
	0.447	0.281	0.049	0.109	0.038	26.9	20.2	5.0	11.5	6.6	0.266	0.752	0.703	0.592	0.488	0.570
	LAR (cm^2g^{-1})					*RLGR* ($cm^2cm^{-2}day^{-1}$)					*SLA* (cm^2g^{-1})					
N_0	140	110	90	77	48	0.325	0.092	0.020	0.004	0.022	341	244	160	190	140	156
	199	153	105	71	52	0.296	0.117	0.006	0.014	−0.036	320	268	180	169	152	162
N_1	160	125	92	82	72	0.338	0.097	0.048	0.022	−0.105	307	285	169	163	242	129
	182	144	110	38	62	0.404	0.084	0.048	−0.010	−0.052	339	287	166	172	163	152
N_2	169	134	101	95	90	0.372	0.183	0.063	0.046	0.005	384	281	179	167	182	165
	210	162	91	88	86	0.432	0.171	0.020	0.039	0.016	326	358	182	163	190	174
N_3	190	139	97	86	88	0.195	0.344	0.093	0.053	0.007	479	310	182	159	164	178
	179	140	129	106	66	0.446	0.230	0.061	0.042	0.013	333	235	175	195	180	132

opposed to those on leaf area, were of relatively short duration. In the present experiment, the effects of *N* on leaf area and dry weight were clearly visible at day 32 after sowing. The increase in leaf area produced by *N* application could be attributed to both an increase in the number of living leaves per plant and the size of the individual leaves (data not shown). Watson (1952) and Arney (1952) also reported similar results.

RGR, *NAR* and *RLGR* were markedly affected by *N* application but the effect of *N* was not so marked on *LAR* and its two components, *LWR* and *SLA*, although the effect of *N* was significant on *LAR* and *LWR* (Table 1). These results might be attributed to the increase in dry matter of the whole plant rather than the increase in leaf area and leaf dry weight resulting from increased *N* level. They are in agreement with the results obtained by Osman et al. (1977), El-Sharer et al. (1979) and El-Hattab et al. (1980). Robson and Parsons (1978) reported that low *N* treated ryegrass had higher *RGR* and *NAR* and lower *LAR* and *SLA* than the high *N* plants, particularly at the end of the experimental period. In the present investigation also *RGR* and *NAR* of N_0 treatment at 39–46 days after sowing had higher values. This may be due to the sampling error and because N_0 treated plants had lower leaf area than the high *N* treated plants, so they received much more light per unit of that leaf area.

All the growth attributes except *LWR* decreased with the advance in plant age, with a sharp reduction of *NAR* of low *N* plants at 46–53 days after sowing. *LWR* increased to the second harvest and after that it gradually decreased. The decrease in these growth attributes could be attributed to self-shading of lower leaves by upper leaves (Thorne 1961). The decrease of *NAR* would also occur if the metabolic activity of the leaves decreased as the plant aged (Beevers and Cooper 1964). the initial establishment of leaf area or the effects of "sinks" elsewhere in the plant could also account for the decline of these growth attributes with time.

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PHYSIOLOGICAL ANALYSIS OF NITROGEN RESPONSE IN RAPE AND TURNIP

II. PHOTOSYNTESIS, RESPIRATION AND LEAF ANATOMY

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The influence of nitrogen (N) on photosynthesis, dark respiration, chlorophyll content, stomatal and leaf anatomical characters of rape and turnip was investigated. The photosynthetic gain, chlorophyll content and the rate of dark respiration increased with the increasing level of N. Stomatal number and stomatal pore length were increased and leaf diffusion resistance was decreased with the increase in N. Leaf thickness and the mean cross-sectional areas of palisade and spongy parenchyma cells did not vary much with increased N. Turnip was less responsive to N in relation to anatomical characters.

Keywords: chlorophyll content, dark respiration, leaf thickness, leaf diffusion resistance, rape and turnip

Introduction

Many experimental results have indicated that nitrogen deficiency leads to a pronounced depression of photosynthetic rate (Tanaka et al., 1966; Dale 1972; Nevins-Loomis 1970; Osman et al. 1977; Robson-Parsons 1978). Since nitrogen plays a dominant role in chlorophyll formation it is not surprising that a very close correlation exists between *N* content and chlorophyll content of foliage.

Many studies have shown an appreciable effect on respiration when *N* was applied to the whole plant or to its detached leaves or roots (Austin 1960; Berner 1971; Singh-Singh 1978; Robson-Parsons 1978). This is due to the fact that more photosynthesis becomes available and the mass of respiring tissue increases with increasing *N* supply.

Nutrient deficiency may induce xeromorphism and reduce the rate of transpiration. The reactivity of stomata may also be influenced and Neuwirth and Fritzsche (1964) emphasized particularly the importance of an adequate and balanced fertilization for securing maximal stomatal reactivity. Similarly Pharis and Kramer (1964) found that the rate of transpiration was increased with *N* supply up to an optimum, but at the highest N content it decreased again.

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The aim of the present study was to investigate the effect of N on photosynthesis, dark respiration and stomatal and leaf anatomical characters of rape and turnip.

Materials and methods

Seeds of forage rape (*Brassica napus* cv. Lair) and turnip (*Brassica campestris* cv. Labra) were sown in 12.5 cm plastic pots in a glasshouse of Welsh Plant Breeding Station, Aberystwyth, U. K. The soil composition was equal parts of unsterilized soil, grit, sand and peat.

Four levels of N were used as follows:

N_0 = No nitrogen
N_1 = 0.4 g $NaNO_3$ per pot (37.5 kg N/ha)
N_2 = 1.6 g $NaNO_3$ per pot (150.0 kg N/ha)
N_3 = 6.2 g $NaNO_3$ per pot (600.0 kg N/ha)

In each pot 8 g "double season PK" (150 kg/ha of P_2O_5 and K_2O were applied. The experiment was arranged as a randomized complete block design with four replications.

The photosynthetic gain was measured, as described by Johnston and York (1971), at 40, 41, 42 and 43 days after sowing. On the same days, chlorophyll content was determined, silicone impressions were made (Sampson 1961) and leaf segments were fixed for anatomical study. Dark respiration rate was measured using Warburg apparatus at 45, 46, 47 and 48 days after sowing. The diffusion resistance of both surfaces of leaves were measured by an automatic porometer (Delt-T devices Model MK 11) at 39 days after sowing. Two plants per treatment per cultivar were sampled from each replication and the third leaves of those plants were used for the above mentioned measurements. The number of stomata of microscopic fields was counted and subsequently converted to the number per mm^2 of leaf. The outlines of 10 randomly selected stomata were drawn on paper and the stomatal pore length was determined and converted to μm. Transverse sections of leaf segments were cut by hand with a razor. The sections were transferred to water, bleached for 1 to 2 minutes in fresh parazone and then rinsed in water before staining with toluidene-blue. They were finally rinsed in water and mounted in glycerol. Sections were projected by a projecting microscope and the outline of the cells of the leaf tissues was drawn on paper. From the outline leaf thickness was determined. Subsequently, the area occupied in the drawings by the palisade and spongy parenchyma cells was determined separately by an automatic area meter. From this the mean cross-sectional areas of palisade and spongy parenchyma cells was estimated by dividing the area of the palisade and spongy parenchyma by the respective number of cells. Data were analyzed statistically.

Results and discussion

Mean squares from the analysis of variance are shown in Table 1. The effect of N was highly significant for all the characters. Cultivar difference was significant for all the characters except number of stomata of the upper surface. Nitrogen *X* cultivar interaction was significant for all the characters except photosynthetic gain, number of stomata (lower) and leaf thickness.

The effect of different levels of N for various characters are presented in Table 2. The photosynthetic gain increased steadily up to the highest level of N in both cultivars. Ryle and Hesketh (1969) and Nevins and Loomis (1970) reported that N supply decreased stomatal resistance, possibly because of the interaction with water, and this could explain its effect on photosynthetic resistance. Since N plays a dominant role in chlorophyll formation it is not surprising that a very close correlation exists between nitrogen content and

chlorophyll content. The present study demonstrated that the chlorophyll content increased with the increasing N level. Several workers reported that the rate of photosynthesis in leaves is determined by the chlorophyll content. Others have failed to find such a correlation. Hesketh (1963) showed that species can vary greatly in the rate of photosyntesis and this variation was not related to chlorophyll content. However, in the present study, the increased photosynthetic gain with added N may partly be explained by the increased chlorophyll content.

The rate of dark respiration increased with increasing level of N. This agrees with the findings reported by other workers (Austin 1960; Schmidt and Blaser 1967; Singh and Singh 1978; Robson and Parsons 1978). This effect seems to be due to the following reasons:

Table 1

Mean squares from analysis of variance of various characters

Item	df	Photosynthetic gain (mg $dm^{-2}h^{-1}$)	Total chlorophyll (mg dm^{-2}) X 10^{-2}	Dark respiration (μl O_2 $min^{-1}mg^{-1}$) X 10^{-5}	Dark respiration (μl O_2 $min^{-1}cm^{-2}$) X 10^{-4}
Replication	3	7.51*	1.86***	0.40	0.29
Cultivar (C)	1	20.52**	118.73***	22.70***	20.30***
Nitrogen (N)	3	172.60***	251.53***	17.60***	64.76***
C×N	3	1.14	7.87***	1.32**	1.26**
Error	21	1.89	0.21	0.17	0.20

No. of stomata per mm^2		Pore length (μ)		Leaf diffusion resistance (s/cm)	
Upper	Lower	Upper	Lower	Upper	Lower
79.36	146.36	0.44	0.53	0.30	0.08
7.03	1471.53***	18.60***	6.12***	5.36***	1.40***
1901.45***	1970.45***	15.66***	13.50***	6.69***	2.93***
203.11*	22.78	1.37*	3.23***	1.03**	0.99***
46.06	79.98	0.34	0.36	0.14	0.078

Leaf thickness (μ)	Area of palisade parenchyma (μ^2) X 10^3	Area of spongy parenchyma (μ^2) X 10^3
17.85*	5.25	0.51
365.52***	563.52***	118.83***
89.91***	721.55***	88.58***
4.72	143.22***	10.31*
4.05	8.72	2.58

*, ** and *** indicate significant at 5%, 1% and 0.1% level, respectively

Table 2

Mean values of various characters as affected by nitrogen and cultivar

Nitrogen level df	Photosynthetic gain (mg $dm^{-2}h^{-1}$)	Total chlorophyll (mg dm^{-2})	Dark respiration (μ 10_2 $min^{-1}mg^{-1}$)	Dark respiration (μ 10_2 $min^{-1}cm^{-2}$)
N_0	6.59	1.042	0.0177	0.0666
	5.82	1.194	0.0211	0.0775
N_1	7.84	1.393	0.0190	0.0797
	6.60	1.684	0.0232	0.0889
N_2	10.62	1.702	0.0214	0.0996
	9.11	2.274	0.0259	0.1265
N_3	12.75	2.153	0.0255	0.1268
	11.85	2.679	0.0346	0.1435

	No. of stomata per mm^2		Pore length (μ)		Leaf diffusion resistance (s/cm)	
	upper	lower	upper	lower	upper	lower
N_0	89	115	15.7	14.2	5.38	3.98
	80	128	14.8	14.6	3.65	2.50
N_1	93	127	16.3	14.7	4.15	2.25
	90	137	15.3	14.6	3.10	2.15
N_2	110	142	18.2	17.0	2.95	2.08
	104	160	16.7	15.6	2.80	2.00
N_3	112	148	20.0	18.2	2.60	1.93
	126	161	16.9	15.6	2.25	1.90

	Leaf thickness (μ)	Area of palisade parenchyma (μ^2)	Area of spongy parenchyma (μ^2)
N_0	263	966	590
	348	1611	762
N_1	315	1437	640
	374	1701	836
N_2	339	1830	834
	394	1911	897
N_3	342	1907	870
	424	1980	928

In each pair: upper value for Lair and lower value for Labra.

(1) nitrate nitrogen accelerated respiration due to the diversion of respiratory NADPH for nitrate reduction and its utilization along with the respiratory intermediates for amino acid synthesis;
(2) N accelerated respiration through the stimulation of synthetic reactions requiring adenosine triphosphate. Moreover, Ohira and Mabuchi

(1958) stated that N deficiency led to variation in cytochrome oxidase activity of the plant and they reported that N deficient shoots and roots of rice seedlings had a significantly lower cytochrome oxidase activity.

Although stomatal number and stomatal pore length of both surfaces rose with the increase of N level, the differences between N_2 and N_3 and between N_0 and N_1 treatments were not significant in most cases. The stomatal pore length of Labra was less affected by N. Arney (1952), however, did not find any appreciable effect of N on stomatal number in kale. The leaf diffusion resistance of both surfaces decreased with increase in N level, although in Labra this decrease was less steep, especially on the lower surface.

Leaf thickness appeared to be less affected by N. SLA (specific leaf area) also did not vary much with N level (Paul 1987). So both results correspond with each other, since greater SLA indicates thinner leaves and vice versa. Arney (1952) also did not find any significant effect of N on leaf thickness in kale.

The mean cross-sectional areas of palisade and spongy parenchyma cells did not vary much with increased N; there was no significant difference between N_2 and N_3 treatments. Labra was less responsive to N in relation to anatomical characters. Therefore, the larger leaf size of the high N leaves was at least partly the result of increased cell size, although increased cell number must play an important role as well.

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EFFECT OF ZINC-ENRICHED CLOVER (*TRIFOLIUM PRATENSE* L.) AND INORGANIC ZINC ON WHEAT

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The relative effectiveness of zinc-enriched clover *(Trifolium pratence* L.) and inorganic Zn applied as zinc-chloride salt was investigated for spring wheat under field conditions. Zinc was applied at 0 and 5 mg kg^{-1} soil rate through both these sources mixed at surface (0—12.5 cm) and sub-surface (12.5—25 cm) layers of the soil in specially designed polythene frames. Both, zinc- enriched clover and inorganic zinc significantly increased the grain yield of wheat and Zn uptake though the former source was relatively more effective than the latter in these respects. Zn-enriched clover was at par whether the application was done at the surface or sub-surface layer for enhancing the yield of wheat, whereas inorganic Zn was better utilized at the surface layer as compared to its application at a lower depth.

Keywords: crop response, clover, wheat, zinc carriers, Zn deficiency

Introduction

Zinc fertilization has become increasingly important for obtaining a high yield of field crops. High sensitivity to zinc deficiency is exhibited by such rops as buckwheat, hop, beet, potatoes and red clover on acid highly podzolized light soils and zinc-poor calcareous and highly humified soils (Yagodin, 1984). Wheat crops, among cereal crops are quite often being cultivated in rotation with fodder legumes and grass-clover mixtures, and help in the fixation of nitrogen and may be used as a green manure. Vez (1972) mentions the particular value of fodder legumes and grass-clover mixtures in this connection. Also, Simon et al. (1981) advise the inclusion of lucerne in rotations and expect and increase in grain yield of upto 10% by including a main forage crop in the rotation if cereals are the first subsequent crops.

For meeting the zinc requirements of crops, several carriers (inorganic, chelated or complexed) of zinc can supply this nutrient. Chesnin (1963) has reported that organic sources of zinc are generally more effective than inorganic sources when banded under the seed. However, not much information

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is available as regards the supply of zinc through organic-bound forms. The present study was, thus, undertaken to assess the relative efficiency of zinc-enriched red clover and inorganic zinc for wheat.

Materials and methods

This study was conducted at the experimental field of the Timiryazev Agricultural Academy, Moscow, USSR. The dernopodzolic soil used in this experiment had a pH 4.25 (KCl extract); the sum of adsorbed bases and hydrolytic acidity 7.86 and 4.77 m. eq per 100 g soil, respectively; the available P_2O_5 and K_2O contents 3.05 and 9.02 mg per kg soil, respectively and the ammonium acetate extractable (Krüpskii-Alexandrova 1964) Zn content was 4.5 ppm. the soil was low in the available zinc supply.

In order to enrich the clover (*Trifolium pratense* L.) with zinc, in a young clover growing field an area of 3×3 sq. m. strip was marked and broadcasted with zinc at 11.2 kg per ha supplied through zinc chloride salt. Also, in the same clover growing field, the same area was marked whereform the clover plant material could be gathered without the application of zinc. Clover plants were grown for a period of 3 months, with and without the application of zinc. Clover plants were then uprooted alongwith roots; washed with ordinary and distilled water and dried. The dried clover plant material was then ground in a stainless steel grinder and the total zonc content of this material, without and with the added zinc, was determined after digesting the plant material in a tri-acid mixture ($HNO_3-HClO_4-H_2SO_4$) and analysing the extracts for total zinc on atomic absorption spectrophotometer. The total Zn content of the clover plant material with and without applied Zn was 345 and 55 ppm, respectively.

For raising the wheat crop under field conditions, iron rod frames measuring $33 \times 33 \times 25$ cm[13] were wrapped in polythene sheets in such a way to have openings at two ends along with the 25 cm measurement. These polythene wrapped frames were then inserted into the soil upto a depth of 25 cm. The soil in these frames (in each frame, the oven-dry weight of the soil up to a depth of 12.5 cm was 15 kg) was treated with Zn-enriched clover and inorganic Zn ($ZnCl_2$) upto two depths i.e. 0.12.5 cm and 15.5 to 25.0 cm. The treatments were designated as follows:

(1) Control
(2) Zn_1 — Zinc supplied through zinc chloride salt equivalent to the level of Zn in $clover_1$.
(3) $Clover_1$ — Plant material having 55 ppm Zn (without added Zn) and the amount of clover added was equal to the amount of $clover_2$.
(4) Zn_2 — Zinc supplied through zinc chloride 5 ppm.
(5) $Clover_2$ — Plant material having 345 ppm Zn (Zn riched) and the amount of $clover_1$ but supplying Zn equivalent to 5 ppm as added through Zn_2 treatment.

Table 1

Details of treatments

Treatments	Added clover (g/frame)*	Zn content of clover (ppm)	Added Zn (mg/frame)*
Control	—	—	—
Zn_1	—	—	11.9
$Clover_1$	217.4	55	11.9
Zn_2	—	—	75**
$Clover_2$	217.4	345	75

* Frame volume $= 33 \times 33 \times 25$ cm; oven dry wt. of soil upto 12.5 cm depth in a frame $=$ $=$ 15 kg

** Zn equivalent to 5 ppm

Further details of these treatment are summarized in table 1. All treatment were given 150 ppm N, 100 ppm P_2O_5 and 100 ppm K_2O supplied through $Co(NH_2)_2$, KH_2PO_4 and KCl respectively. Fifty plants of spring wheat (var. S. aratskaya-29) were raised in each frame which were thinned to 35 after 10 days. The experiment was laid out according to the randomized block design having three replications. At maturity, the yield of wheat was recorded and plants analysed for the total Zn content (after digestion in a tri-acid mixture, $HNO_3 - HClO_4 - H_2SO_4$) on an atomic absorption spectrophotometer.

Results and discussion

Grain yield of wheat as influenced by the application of clover, inorganic Zn and Zn-enriched clover applied at two soil depths is shown in Fig. 1. Zn-enriched clover ($clover_2$) and inorganic zinc (Zn_2) significantly increased the grain yield of wheat, although Zn-enriched clover proved relatively better than inorganic Zn. The increase in the grain yield of wheat over control was 84% with Zn-enriched clover and 61% with inorganic Zn when applied at the surface layer (0–12.5 cm). The increase in the grain yield over control was 81% and 43% with these treatments, respectively, when applied at sub-surface layer (12.5–25.0 cm). This shows that organically bound Zn when applied at surface or sub-surface layer had almost the same effect, whereas application of inorganic Zn (Zn_2) at surface layer had an advantage when applied at a lower depth.

The uptake of Zn by wheat grain as shown in Fig. 2 for the surface and sub-surface application of various treatments indicated significant increase

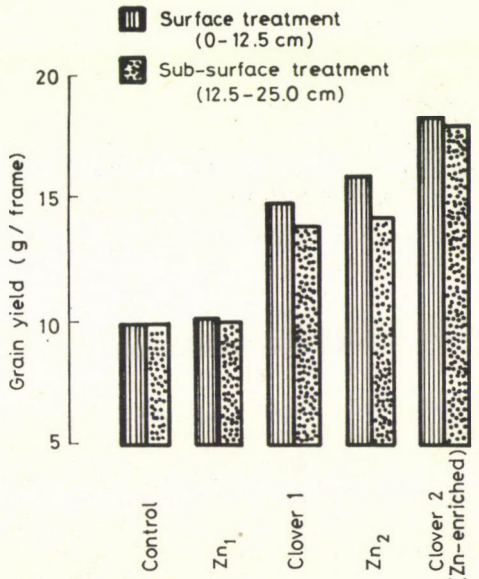

Fig. 1. Grain yield of wheat as influenced by Zn-enriched clover and inorganic Zn (LSD at 0.05 for Zn sources, 5.0; for depth of treatments, NS)

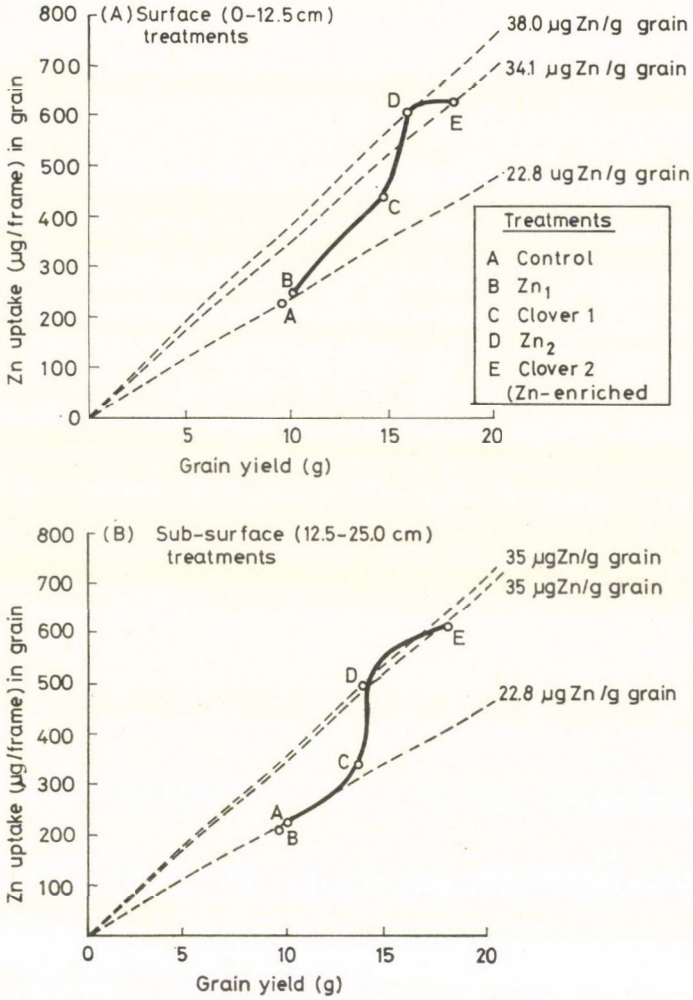

Fig. 2. Grain yield of wheat and Zn uptake in wheat grain as influenced by Zn-enriched clover and inorganic Zn applied at surface and sub-surface layers of soil

from Zn-enriched clover ($clover_2$) and inorganic zinc (Zn_2) over control. The percent increase in Zn removal by wheat grain over control was 175 and 167 with $clover_2$ and Zn_2 treatments, respectively when these treatments were applied at surface. With the application of these treatments at the sub-surface layer, the percent increase in Zn removal was 177 and 120 over control with $clover_2$ and Zn_2 respectively. Thus Zn uptake was almost the same, whether the organically bound Zn is applied at the surface or at the sub-surface layer and in case of inorganic form of Zn, its removel was relatively higher when applied at the surface layer.

The Zn-enriched clover proved more effective in enhancing the yield of wheat and Zn uptake than inorganic zinc at the surface as well as sub-surface application. At the same time, there was also a possibility that apart from Zn some other constituents of clover were effective in enhancing the growth of wheat. Because from Zn-enriched clover, the plants with 34.1 μg Zn per g grain grew a little more (as evidenced from the grain yield) than did the plants with 38.0 μg Zn per g grain from inorganic zinc (Fig. 2A). Similarly these treatments when applied at sub-surface layer (Fig. 2. B), again from Zn-enriched clover (clover$_2$), plants with 35 μg Zn per g grain made a more growth than did the plants with 35 μg Zn per g grain desired from inorganic zinc (Zn_2).

Conclusions

The practical utility of this study suggests that when fodder legumes and grass-clover mixtures are grown in rotation with wheat crop, soil application of zinc to the grass-clover mixtures, in the zinc deficient areas, ensures and adequate supply of zinc not only to the grass-clover mixtures but also to the following wheat crop, if these grass-clover mixtures are used as a fodder or green manure. Zinc present in the organically-bound forms is liberated during the breakdown of the grass-clover mixtures, when ploughed as a green manure and becomes available in the vicinity of the roots of wheat. The lower efficiency of direct application of inorganic zinc to wheat can be ascribed to its retrogradation into reaction products that are sparingly soluble (Kalbasi et al., 1978; Sinha et al. 1975) or to losses of inorganic zinc through leaching.

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INTERACTIVE EFFECTS OF SOIL MOISTURE CONTENT AND HORMONAL TREATMENT ON DRY MATTER AND PIGMENT CONTENTS OF SOME CROP PLANTS

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The fresh and dry matter yield of leaves, stems and roots of maize, cowpea and broad bean plants were sharply lowered with the decrease of soil moisture content. Treatments with any of the three phytohormones; IAA, GA_3 or kinetin resulted in a considerable increase in fresh and dry matter, whatever the soil moisture content used. Similarly a considerable increase in pigment contents was recorded in hormone treated plants in comparison with those of plants subjected only to the corresponding levels of soil moisture content, whatever the phytohormone used and plant cultivated.

Keywords: maize, cowpea, broad bean, Zea mays, Vigna sinensis, Vicia faba, hormonal treatment, IAA, GA_3, kinetin, soil moisture, yields

Introduction

It is a common knowledge that water deficit has an adverse effect on the growth rate of plants and consequently on final yield (e.g. Hsiao et al. 1976; Babalola and Fawusi, 1980; Imamul-Huq and Larher, 1983; Pandey et al., 1984; Bejaoui, 1985). There is also some evidence that drought impedes the biosynthesis of photosynthetically active pigments (Prisco and O'Leary, 1972; Rasmussen, 1976; Kaiser et al. 1983). These alterations in plant growth and pigments could be due to a decrease in natural growth hormones in plant tissues (Shah and Loomis, 1965; Itial et al., 1978; Walker and Dubroff, 1981.) In accordance with this, Browning (1973) found that in *Coffea arabica* plants, which have previously been subjected to water stress, the endogenous cytokinin level in the xylem sap was again elevated after irrigation. Thus the aim of the present work was to test the effect of exogenous treatments with the phytohormones; Indole acetic acid, Gibberellic acid or kinetin in counteracting the adverse effects of drought on growth and the photosynthetic pigments of maize, cowpea and broad bean plants.

Materials and methods

Grains of maize *(Zea mays)* and seeds of cowpea *(Vigna sinesis)* and broad bean *(Vicia faba)* were sowed in plastic pots containing 2 Kg air dried soil. The seedlings were left to grow under the desired soil moisture content (100%, 90%, 70%, 50% and 30%) until having two foliage leaves. Then they were sprayed with an aqueous solution (50 ppm) of any of the three phytohormones; IAA, GA_3 or kinetin. During the experimental period, the photosynthetic pigments (chlorophyll — a, chlorophyll — b and carotenoids) were spectrophotometrically determined. Fresh and dry weight of different plant organs were determined at the end of the experimental period.

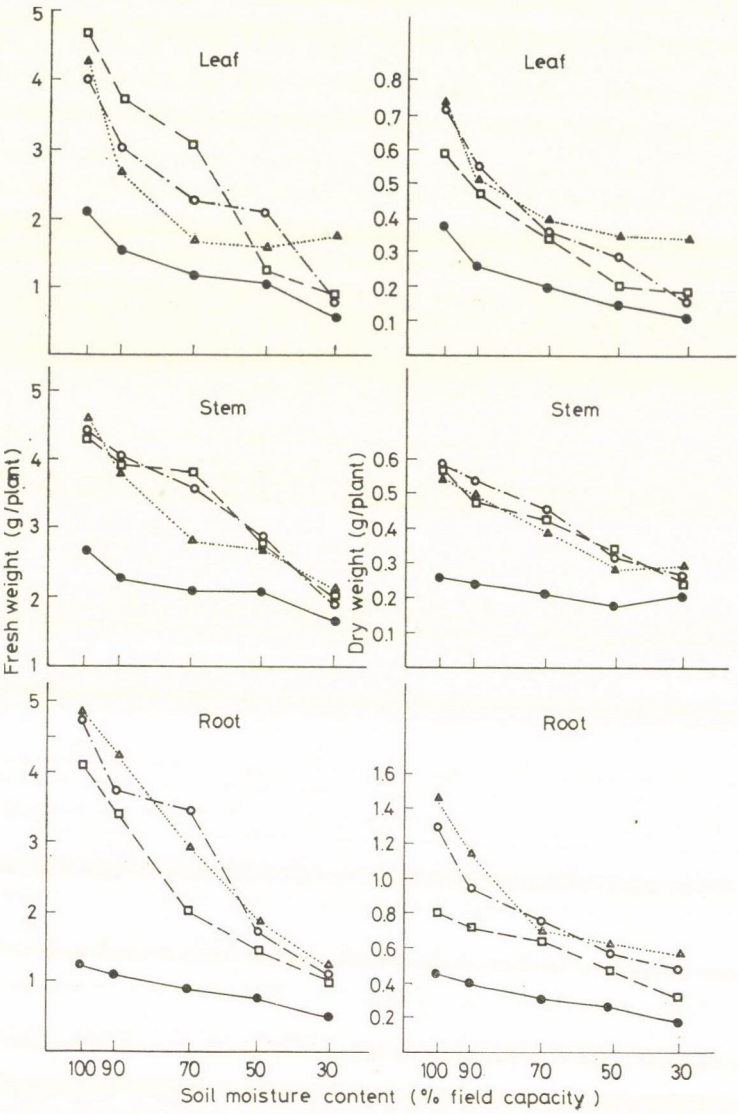

Fig. 1. Interactive effect of soil moisture contents and phytohormonal treatments of fresh and dry weight of maize plants

●——● untreated □— — —□ IAA ○—·—·—○ GA_3 △— — —△ Kinetin

Results and discussion

Fresh and dry weights of leaves, stems and roots of each of the three experimental plants were sharply reduced with the decrease of soil moisture content. Phytohormonal treatments with any of the three phytohormones; IAA, GA_3 or kinetin resulted in a censiderable increase in fresh and dry weights of the three experimental plants, whatever the soil moisture content used (Figs 1, 2 and 3).

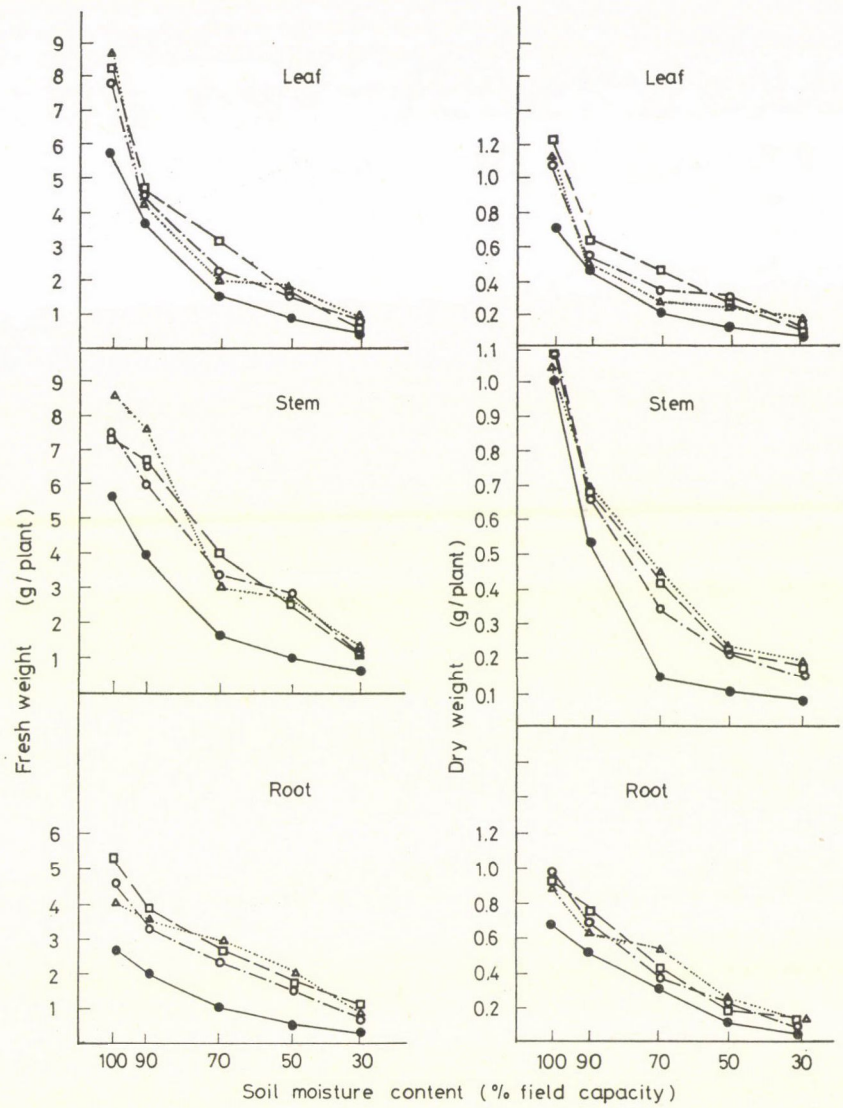

Fig. 2. Interactive effect of soil moisture contents and phytohormonal treatments on fresh and dry weight of cowpea plants

●——● untreated □— — —□ IAA ○—·—·—○ GA_3 △— — —△ Kinetin

This observed increase in fresh weight of drought stressed plants after hormonal treatment may indicate that the growth hormones used; IAA, GA_3 or kinetin had increased the plant efficiency of water uptake, conservation and utilization. The promotion in dry matter production could be attributed to a rapid increase in cell division, cell enlargement and accumulation of building units (Kessler, 1961; Shah and Loomis, 1965; Sinha, 1969; Singh and

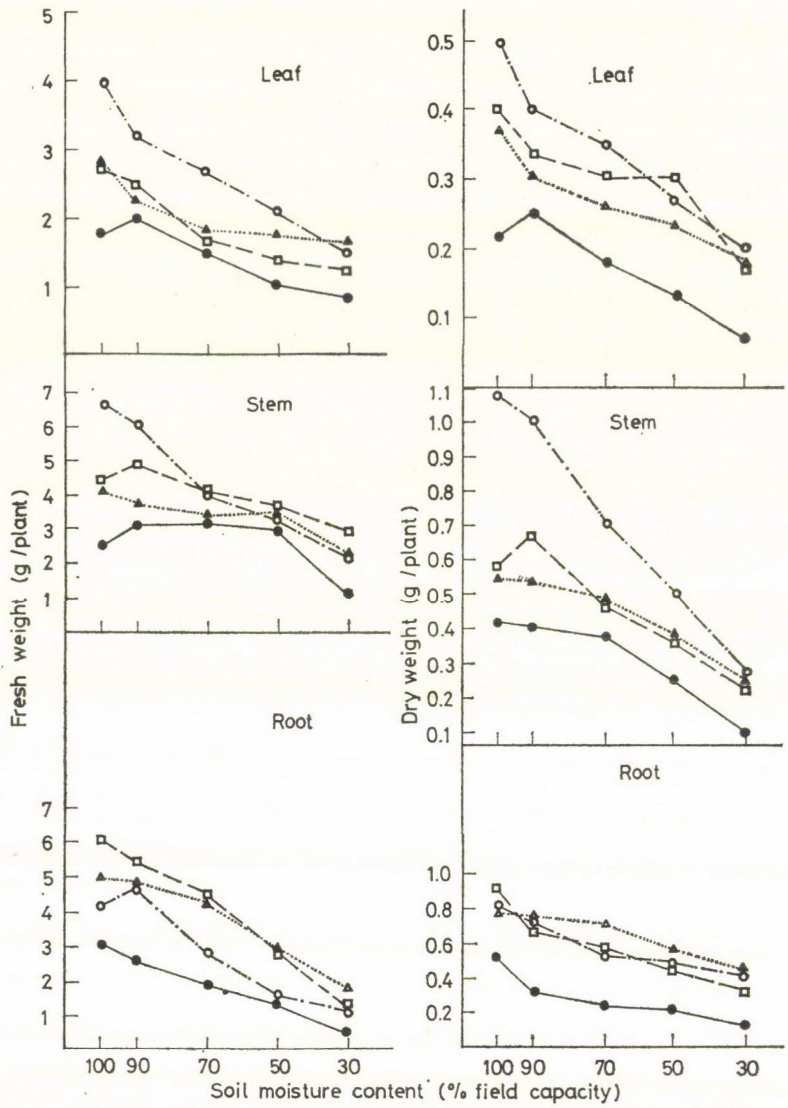

Fig. 3. Interactive effect of soil moisture contents and phytohormonal treatments on fresh and dry weight of broad bean plants

●——● untreated □—·—·—□ IAA ○—·—·—○ GA_3 △ — — —△ Kinetin

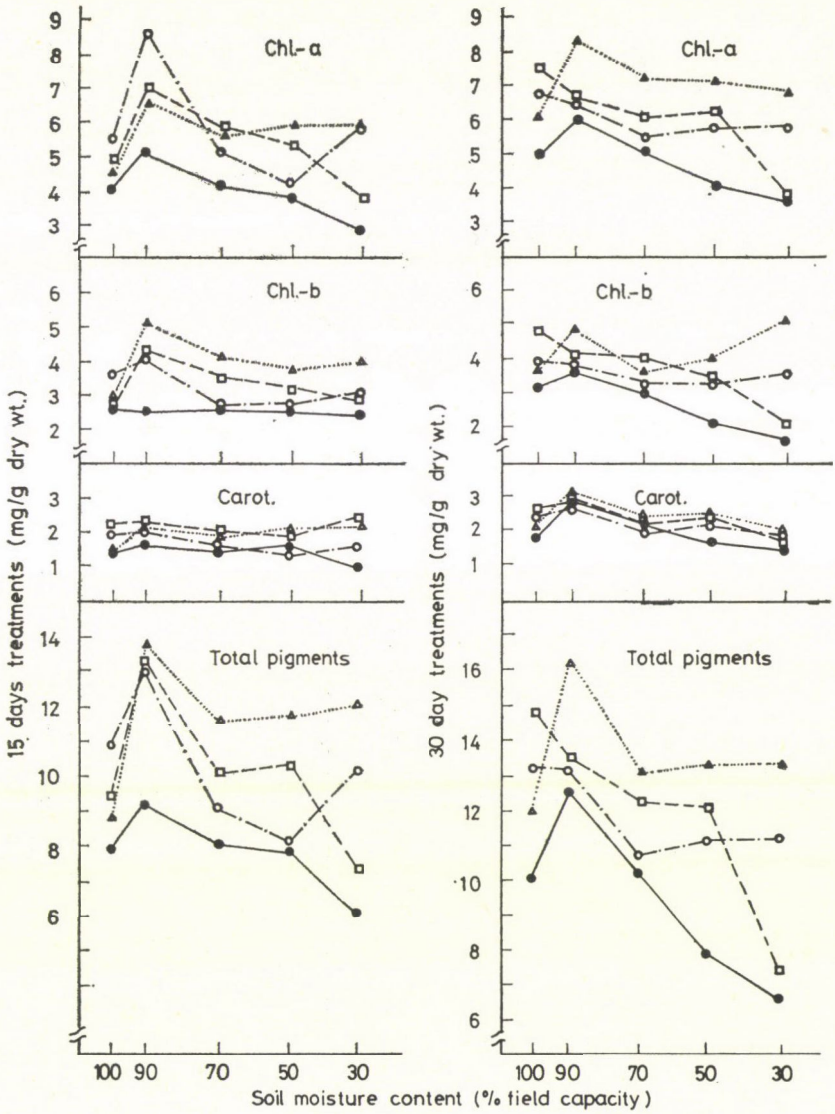

Fig. 4. Interactive effect of soil moisture contents and phytohormonal treatments on pigment contents in leaves of maize plants

●——● untreated □—·—·—□ IAA ○—·—·—○ GA_3 △ — — —△ 1 Kinetin

Darra, 1971). This means that treatment with any of the three phytohormones could alleviate the adverse effects, of at least moderate drought stress, on the growth of maize, cowpea and broad bean plants.

Concerning the photosynthetic pigments, it can be seen that in maize leaves, the maximum contents of photosynthetic pigments were obtained in plants subjected to the level of 90% soil moisture, while the minimum contents

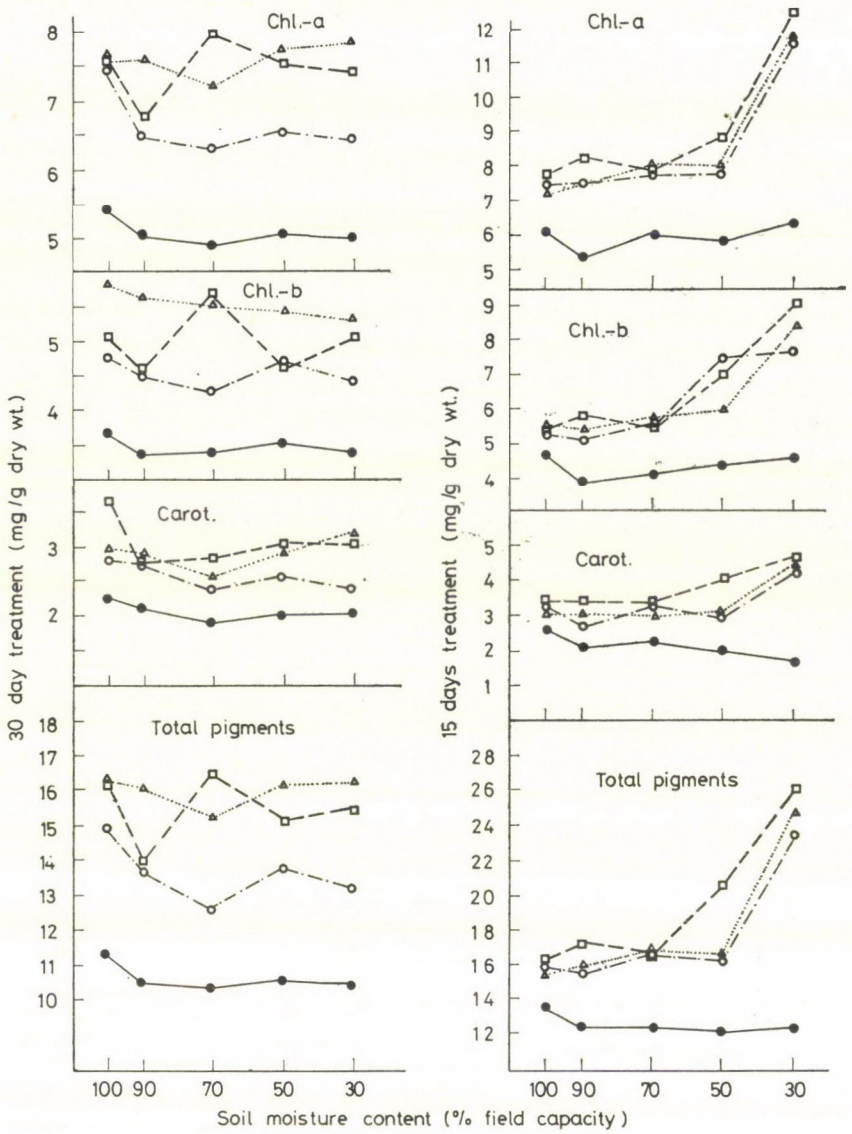

Fig. 5. Interaction effect of soil moisture contents and phytohormonal treatments on pigment contents in leaves of cowpea plants

●——● untreated □—·—·—□ IAA ○—·—·—○ GA_3 △— — —△ Kinetin

were in plants subjected to the level of 30% soil moisture content, whatever the period of treatment used (Fig. 4). In cowpea and broad bean plants, there were no considerable differences in the pigment fractions and consequently in the total pigment contents of plants subjected to the different soil moisture levels, whatever the period of treatment (15 or 30 day) used (Figs 5, 6).

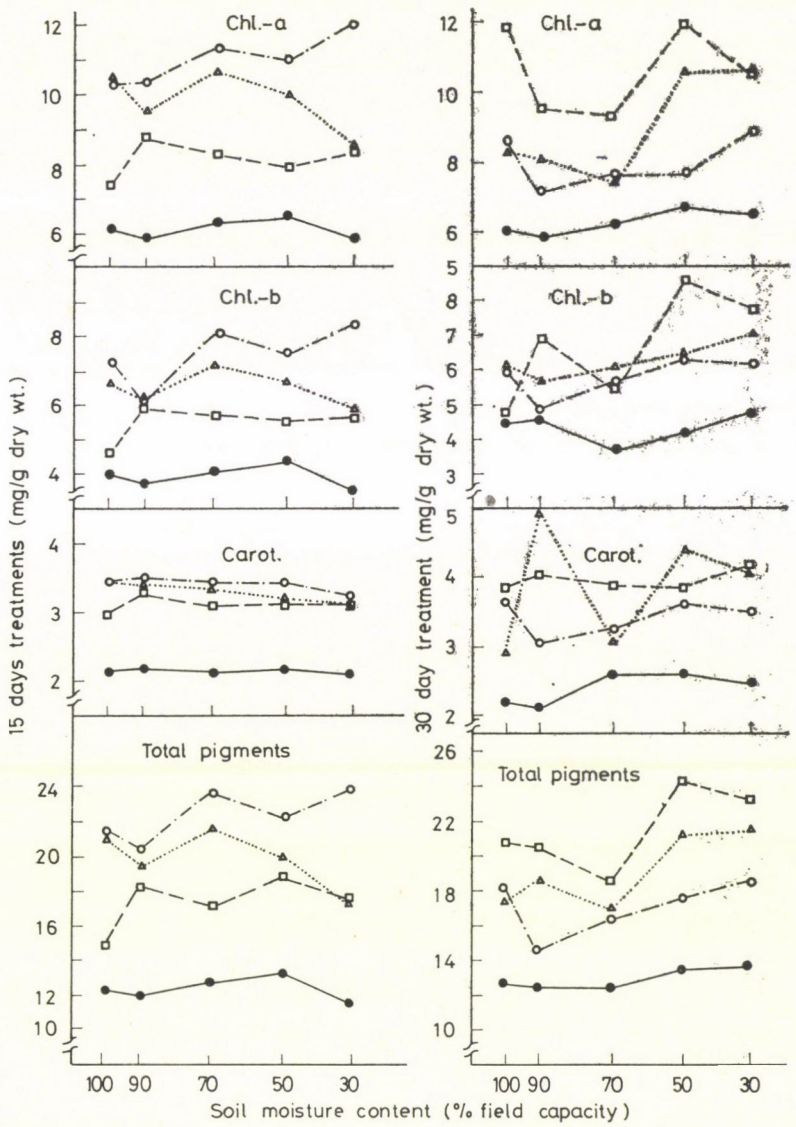

Fig. 6. **Interactive effect of soil moisture contents and phytohormonal treatments on pigment contents in leaves of broad bean plants**

● untreated □ IAA ○ GA_3 △ Kinetin

Spraying with any of the three phytohormones; IAA, GA_3 or kinetin, induced a considerable increase in pigment contents in comparison with those of plants subjected only to the corresponding levels of soil moisture contents, whatever the period of treatment was, plant type cultivated and phytohormone used.

This stimulatory effect on pigments' biosynthesis after exogenous hormonal treatments is in agreement with the results obtained by some other authors (Varshney and Baijal, 1979, Banerji, 1979; Shaddad and Heikal, 1982) who indicated the involvement of exogenously applied phytohormones in the pigment biosynthesis of salt stressed plants. Such enhancement was also recorded in normally cultivated plants after being sprayed with any of the three phytohormones (Pilet and Hofer, 1966; Paranjothy and Wareing, 1971) which could be attributed to the inhibition of chlorophyll degradation. Other authors attributed this promotion in chlorophyll synthesis to the stimulation of chlorophyll-(ide) synthesis (Stobart et al., 1972; Bengston et al., 1977; Sabater and Rodriquez, 1978). Therefore it can be said that under drought stress, the levels of the naturally synthesized hormones are probably suppressed and hence the exogenous application of phytohormones supplies additional quantities, which are implicated in pigment and growth promotion.

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INTERACTION EFFECT OF Fe AND Mn ON GROWTH AND NUTRIENT CONTENT OF MOONG (*PHASEOLUS AUREUS* L.)

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The effect of Fe and Mn fertilization on the dry matter yield, their content and uptake in moong, grown in an alkaline soil, was studied in a greenhouse experiment. There were four levels of Fe and Mn viz. 0, 25, 50 and 100 ppm. The dry matter yield increased significantly with the application of 25 ppm Fe, but it increased non-significantly with Mn application, the highest yield was observed with the application of 25 ppm Fe and 50 ppm Mn, and further application of either Fe or Mn decreased the yield significantly. An increase in the supply of Fe, decreased Mn content and its uptake and result in significant decrease in dry matter yield. Fe—Mn disorder was better related with Fe/Mn concentration ratio in soil and plants than either with Fe or Mn content of the plant and soil. The value of Fe/Mn concentration ratio of 0.92 in soil and 6.45 in plant tissue was found to be critical above which a significant reduction in yield resulted.

Keywords: Fe—Mn interaction, moong

Introduction

The availability of iron and manganese in soil is regulated by the oxidation — reduction conditions. Soil conditions conductive for oxidation cause marked depression in availability. As such, Mn deficiency is commonly observed in crops grown on neutral and alkaline soils was overcome by the application of ferrous sulphate and suggested that this compound, by virtue of its reducing properties, can effectively correct Mn deficiency in soils containing appreciable quantities of higher oxides of Mn. However, Chinnery and Harding (1980) observed that the high supply of Fe depresses the absorption of Mn leading to reduction in Mn concentration in plant tissue. Sideris and Young (1949) noticed that the high levels of Mn supply reduced the Fe concentration in plant tissues. Swarup and Misra (1972) on the contrary found synergetic interaction effects of Fe and Mn uptake in oats. Thus there are conflicting reports about the effects of excessive doses of Fe and Mn additions on the plant growth. The present investigation studied the effect of Fe and Mn application on the growth and its uptake in moong (*Phaseolus aureus* L.) grown in an alkaline soil.

Materials and methods

A greenhouse experiment was conducted using a Fatehpur loamy sand. The soil belongs to great group Ustipsamments. The soil had pH 8.6 and electrical conductivity 0.3 mmhos cm^{-1} in 1 : 2 soil water suspension. The content of calcium carbonate and organic carbon were 0.65 and 0.32%. Available P and K were 10 and 152 ppm respectively. The DTPA extractable (Lindsay and Norvell 1978) Fe and Mn were 4.1 and 1.6 ppm.

Polythene lined earthen pots were filled with 3 kg soil. The treatment comprised four levels each of Fe and Mn (0, 25, 50 and 100 ppm) applied as $FeSO_4 \cdot 7H_2$ and $MnSO_4 \cdot H_2O$. All the pots received a basal application of 10 ppm N and 32 ppm P_2O_5 as calcium ammonium nitrate and super phosphate respectively. These were repeated three times in a completely randomized design. Moong was grown as a test crop. The crop was harvested after 40 days of germination. The representative soil samples were drawn from each pot after the harvest of crop.

The plant samples were washed successively with 0.1 N HCl, distilled and deionized water, oven dried, weighed and ground in a Willey Mill to pass a 40 mesh stainless steel screen. The plant samples were treated with nitric-perchloric-sulphuric acid mixture. The soil samples were analysed for available Fe and Mn by extraction with DTPA solution (0.005 M diethylene trimine penta acetic acid + 0.1 M triethanolamine + 0.01 M $CaCl_2$, with a pH 7.3) using a soil solution ratio of 1 : 2 and a shaking time of 2 hours. The content of Fe and Mn in the plant and soil extracts was measured by atomic absorption spectrophotometry.

Results and discussion

Yield:

In the absence of added Mn, the dry matter yield increased significantly with the application of 25 ppm Fe and beyond this it decreased. The decrease over the control treatment was significant when Fe was applied at the rate of 100 ppm. Whereas, in the absence of added Fe, the dry matter yield increased non-significantly with the application of up to 50 pppm Mn (Table 1). This suggested that the soil was more deficient in available Fe than in Mn. The highest yield was observed with the application of 25 ppm. Fe and 50 ppm Mn. There was 47.3% reduction in yield over this level with the application of 100 ppm either Fe or Mn. The mean yield increased with the application of 25 ppm Fe and thereafter it decreased sharply. The reduction in mean yield over the control treatment was 33% with the application of 100 ppm Fe. Similarly the average response of Mn was observed up to 50 ppm Mn and further addition of Mn significantly decreased the yield. There was a significant antagonistic effect of Fe application on Mn availability of plants. Gupta (1972) reported 50% reduction in barley yield with the application of 400 ppm Fe. Singh and Yadav (1980) also reported that the higher dose of Fe application significantly decreased the yield of sorghum.

Fe content and its uptake

There was a significant increase in Fe content in plants with the application of either Fe or Mn. Fe uptake was also found to be significantly increased with the application of up to 50 ppm Fe or 25 ppm Mn and thereafter it de-

Table 1

Effect of Fe and Mn fertilization on the dry matter yield, their content in plants and soil

Treatments (ppm)		Dry matter yield (g/pot)	Nutrient content in plant (ppm)		Nutrient uptake in plant (mg/pot)		Soil available nutrient (ppm)	
Fe	Mn		Fe	Mn	Fe	Mn	Fe	Mn
0	0	4.0	318	33	1.26	0.13	4.0	1.7
25	0	4.6	350	40	1.62	0.18	4.8	1.8
50	0	3.9	372	36	1.44	0.14	7.2	2.0
100	0	3.4	401	28	1.36	0.09	9.2	2.3
0	25	4.3	368	72	1.60	0.31	4.4	4.6
25	25	5.1	393	76	2.02	0.39	6.0	4.2
50	25	4.5	440	68	1.98	0.30	8.0	4.0
100	25	3.2	444	54	1.42	0.17	8.4	3.5
0	50	4.5	406	88	1.84	0.40	4.2	6.4
25	50	5.7	456	90	2.60	0.51	5.6	6.2
50	50	3.4	518	72	1.76	0.24	7.6	5.8
100	50	3.0	507	58	1.52	0.17	8.2	5.2
0	100	3.8	443	134	1.68	0.51	5.8	8.4
25	100	3.0	493	114	1.48	0.34	4.6	8.0
50	100	2.5	543	83	1.36	0.20	6.8	7.2
100	100	1.7	528	75	0.92	0.13	7.9	7.0
LSD at P = 0.05								
Fe		0.6	86	6	0.32	0.05	2.6	NS
Mn		0.6	86	6	0.32	0.05	NS	1.8
Fe×Mn		1.2	NS	12	0.64	0.10	5.2	3.6

NS = non-significant

creased sharply. At highest rate of 100 ppm. Jerald and John (1966) also reported that the uptake of Fe was enhanced up to a certain point and then decreased with increasing Mn concentration. Kuo and Mikkelsen (1981) observed that the total uptake of Fe markedly reduced with higher levels of applied Mn in sorghum.

Mn content and its uptake

The Mn application increased the Mn content and its uptake in plants, whereas the Fe application reduced it. The decrease in Mn uptake with the application of 100 ppm Fe was 58.8%. With the increase in Fe content in soil, the plant Fe also increased and it results in decreased Mn content in plants.

As the DTPA available Fe in soil increased from 4.0 ppm in control treatments to 9.2 ppm with Fe treatment, the plant Mn content decreased from 33 ppm in control treatment to 28 ppm with 100 applied Fe. Sommers and Shieve (1942) were of the opinion that higher concentration of soluble Fe in the tissue are invariably associated with low concentration of soluble Mn and vice-versa. Singh and Pathak (1968) reported that the concentration and uptake of Mn in oat decreased significantly with an increased amount of applied Fe. The decrease in concentration and uptake was mainly due to the antagonistic effect between Fe and Mn. Alam (1985) also found a decreased Mn content in rice plants when Fe was applied beyond 30 ppm.

Available Fe and Mn in soil

Iron: At each level of Mn application, increasing rates of Fe application though markedly and successively increased the DTPA available Fe, yet a significant increase occurred at 50 ppm rates of Fe application. At each level of Fe application increasing rates of Mn application caused a slight and non-significant increase or decrease in DTPA extractable Fe.

Manganese: At each rate of Fe application, increasing rates of Mn application generally increased the available Mn significantly and successively. But the increasing rates of Fe application resulted in a non-significant decrease in available Mn. These results indicated that Fe—Mn interaction did not occur in soil.

Prediction parameters of Fe—Mn disorder in plants

Fifty ppm Fe and 20 ppm Mn have been reported to be the critical levels in plants (Jones, 1972). In the present study, neither Fe/Mn disorder nor its correction was correlated with the changes in Mn content in the plants, since the significantly low yield was noticed at a high level of Mn in plants. The Mn concentration in soils and plants as well as Fe concentration in soil were non-significantly correlated with dry matter yield (Table 2). However, the Fe content in plants was significantly correlated with yield ($r = 0.609^*$). Therefore an attempt was made to determine the coefficient of correlations between dry matter yield and Fe/Mn concentration ratios in soil and plants. There was a significant correlation between dry matter yield and Fe/Mn concentration ratios in soil ($R = -0.503^*$) and the plants ($r = 0.498^*$). This suggests that Fe—Mn disorder in plants could be monitored best from Fe/Mn concentration ratios rather than their concentrations. The statistical model of Cate and Nelson (1971) was followed to determine the critical Fe/Mn concentration ratios in soils and plants below which a significant increase in yield could be expected. These values were 0.92 in soil and 6.43 in plants. The present study

Table 2

Coefficient of correlations between dry matter yield and nutrient content in soil and plants

Parameter	Coefficients of correlation (r)
Soil	
Fe	−0.254 NS
Mn	−0.307 NS
Fe/Mn ratio	−0.503*
Plant	
Fe	−0.609*
Mn	−0.009 NS
Fe/Mn ratio	−0.498*

* Significant at 5% level, NS = Non-significant

suggested that the higher rates of Fe application has an antagonistic effect on Mn availability in plants, whereas Mn application has little effect on Fe availability in moong.

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Plant cultivation

FERTILIZATION OF GRASSLANDS WITH VARIOUS RATIOS OF LEGUMES

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The N-fertilization of grass mixtures has potential, ecological and economic limits. Grasslands with various ratios of legumes have to receive differentiated N-fertilization. To grasslands with a higher ratio of legumes the N-fertilization can be restricted if we want to keep the legumes in the grass mixture and also to utilize the nitrogen of the air. According to the result of the experiment the N-fertilizer is reasonably used in a quantity below 150 kg/ha.

Without fertilization the grasslands richer in leguminous plants gave higher yields and nutrient outputs by fixing the atmospheric nitrogen than the grass mixture did (32—58= yield surplus; 54—107% N, 37—72% P_2O_5, 30—55% K_2O, further, 42—97% Ca and 54—119% Mg surplus nutrient).

The N-fertilizer increased the yield of mixed grasslands but at the same time displaced the legumes, so by the 4th year the plant stands of the treatments given high rates of N-fertilization became identical in the different mixtures both as regards the level of yield and the value of components.

PK fertilization is not able to prevent the increasing N-fertilizer doses from displacing the legumes from the grassland.

Keywords: atmospheric nitrogen, components, fertilization, grassland, grassland composition, yield

Introduction

Owing to the increased rate of N-fertilization the leguminous plants have recently been losing territory in the grasslands: their share has decreased or has completely disappeared. Some authors suggest the cultivation and intensive N-fertilization of a single grass species or a grass mixture without legumes, since the grasses react better to the N-fertilizer, and a large dosis of N anyway drives out the legumes from the grassland.

The adverse effect of a high rate N-fertilization on the components, as well as the seasonal shortage and considerably increased price of the N-fertilizer have turned the attention again to grasslands richer in legumes, owing partly to the higher nutritive value, partly to the use of the atmospheric nitrogen and to saving of fertilizer and cost, respectively.

In a number of experiments it was found that in response to high rates of N-fertilization the proportion of legumes decreased in the grasslands (Bánszki 1973, Lorenzo and Labayen 1980). When using one-sided P and K fertiliza-

tion Siscsenkó (1963) and Harmati (1966) experienced an increase in the number of legumes.

According to the research result of Doll, Hatfield and Todd (1961) the N-fertilizer did not increase the yield of the grass-legume pasture, while in the pasture established mostly with grasses a considerable yield increase was achieved. This was confirmed by Schechtner and Deutsch (1966) who found that the lower the number of legumes in the grassland was the higher the yield-increasing effect of N-fertilization was.

Mixed grasslands containing leguminous plants are superior to the grasses both in yield and quality (Lehmann and Bachmann and Guyer 1974). Those mixed grasslands in which legumes are represented in 25–30% (Blagovesenszkaja and Glezina 1977) exceed the grass mixture in quantity and feed value (In: Nagy 1978).

In the German Democratic Republic (Lüdecke 1976) with a 40% share of legumes in the grasslands the optimum rate of N is 120–150 kg/ha, and under irrigated conditions 170–200 kg/ha. According to Reid (1975) to produce 15 t/ha dry matter 360 kg/ha N is needed in the case of grasses, and 180 kg/ha N when grasses and legumes are used in combination (In: Nagy 1978).

Material and methods

The experiment was carried out on the Hajdúszoboszló Trial Grounds of the Debrecen University of Agricultural Sciences in 1982–1985. The soil was chernozem, the analysis data of the 0–20 cm soil layer before the experiment were: pH (KCl) 5.8 K_A 40, total salt % 0.02, $CaCO_3$% 0, humus % 2.7, nutrient content in mg/kg: $NO_3 + NO_2$ 3.6, P_2O_5 79, K_2O 207, Mg 445.

During the years of the experiment the amount of precipitation was 30% less than the 50-year average, and this lessened the effectiveness of the fertilizers.

Table 1

Results of fertilizing grasslands

Number	Treatment			Dry matter output			
	N	P_2O_5	K_2O	grass mixture			
	kg/ha			t/ha	%	D	H
1.	—	—	—	6.55	100	—	79
2.	150	—	—	13.11	200	6.56	131
3.	300	—	—	15.75	240	9.20	149
4.	—	50	150	6.77	106	0.22	79
5.	—	100	300	6.68	102	0.13	78
6.	150	50	150	13.68	209	7.13	134
7.	150	100	300	13.78	210	7.23	135
8.	300	50	150	16.33	249	9.78	151
9.	300	100	300	16.59	253	10.04	152
LSD 5%				1.41	22		

The composition of the mixtures: mixtures containing 0 (grass mixture), 20- and 40% legumes were prepared from the same components. The grasses were: meadow fescue, blue grass red fescue, Hungarian brome grass, dactylis and timothy. The legumes were: white clover and bird's foot trefoil.

The treatments are contained in Table 1.

The laboratory examinations were performed by an ICP apparatus with the methods of the Ministry of Agriculture and Food.

Results and discussion

Four-year average results of the experiments are given in Table 1.

Legume ratio. On the average of 4 years in mixed grasslands planted with legumes 18% and 32% ratio of legumes developed in the control. The different fertilizer treatments provided a varying percentage of legumes. The N-fertilizer pressed back the legumes partly or fully in the mixed grasslands. In the last years of the experiment leguminous plants were no longer found in the grasslands.

PK fertilizers used by themselves did not increase the share of legumes compared to the control (in spite of the moderate PK status of the soil); PK fertilizers used in combination with N were not able to counteract only to slightly moderate the suppressing effect of N on legumes.

Dry matter production. The volume of yield was determined by the N-fertilizer, and that significantly, the PK fertilizers had no essential influence on the yield.

With the grass mixtures (0% legume) the yield of the control was low, but the N-fertilizers resulted in large yields or even in yield surpluses (100–153%). In the case of mixed grasslands with an increase in the share of legumes

with various ratios of legumes (1982-1985)

Grass height cm (H)					and legume ratio %				
18% ratio of legumes					32% ratio of legumes				
t/ha	%	D	H	leg.%	t/ha	%	D	H	leg.%
8.64	100	—	79	18	10.32	100	—	88	32
13.47	156	4.83	127	4	13.93	135	3.61	129	10
14.65	170	6.01	134	2	15.89	154	5.57	138	5
8.68	100	0.04	73	16	10.69	104	0.37	88	28
8.80	102	0.16	76	15	10.76	104	0.44	87	28
13.91	161	5.27	132	5	14.65	142	4.33	132	10
14.27	165	5.63	130	6	14.97	145	4.65	131	8
15.72	182	7.08	142	2	16.40	159	6.08	140	6
16.14	187	7.50	144	1	17.07	165	6.75	142	5
1.54	18				1.54	15			

Table 2

Changes in the nutritive elements of grasslands with various ratios of legumes in response to fertilization (1982–1985)

Elements		Treatments			Values of nutrients as a % of dry matter		
		N	P_2O_5	K_2O	Grass mixture	18%	32%
		kg/ha				ratio of legumes	
N%	1.	—	—	—	1.62	1.89	2.13
	2.	150	—	—	1.99	2.07	2.15
	3.	300	—	—	2.23	2.27	2.33
	4.	—	50	150	1.71	1.99	2.11
	5.	—	100	300	1.74	1.94	2.12
	6.	150	50	150	1.89	2.02	2.14
	9.	300	100	300	2.19	2.20	2.26
P%	1.	—	—	—	0.24	0.25	0.26
	2.	150	—	—	0.22	0.24	0.24
	3.	300	—	—	0.21	0.23	0.24
	4.	—	50	150	0.24	0.26	0.26
	5.	—	10	300	0.25	0.27	0.27
	6.	150	50	150	0.23	0.26	0.26
	9.	300	100	300	0.25	0.26	0.26
K%	1.	—	—	—	2.23	2.21	2.20
	2.	150	—	—	2.11	2.09	2.08
	3.	300	—	—	2.10	2.09	2.09
	4.	—	50	150	2.26	2.24	2.22
	5.	—	100	300	2.31	2.29	2.22
	6.	150	50	150	2.40	2.36	2.34
	9.	200	100	300	2.59	2.54	2.49
Ca%	1.	—	—	—	0.52	0.56	0.65
	2.	150	—	—	0.46	0.47	0.49
	3.	300	—	—	0.44	0.45	0.47
	4.	—	50	150	0.49	0.57	0.63
	5.	—	100	300	0.42	0.47	0.47
	6.	150	50	150	0.42	0.47	0.47
	9.	300	100	300	0.45	0.45	0.48
Mg%	1.	—	—	—	0.18	0.21	0.25
	2.	150	—	—	0.21	0.20	0.23
	3.	300	—	—	0.23	0.19	0.22
	4.	—	50	150	0.18	0.19	0.24
	5.	—	100	300	0.18	0.20	0.25
	6.	150	50	150	0.19	0.20	0.20
	9.	300	100	300	0.20	0.20	0.20

Table 3

Fertilization results of a grass mixture and of grasslands with various ratios of legumes
(1982–1985)
(Percentage values compared to the grass mixtures)

Number	Treatment			Grass mixture	18%	32%
	N	P_2O_5	K_2O		ratio of legumes %	
	Kg/ha					
			% of dry matter output			
1.	—	—	—	100	132	158
6.	150	50	150	100	102	107
9.	300	100	300	100	97	103
			% of yield surplus			
6.	150	50	150	100	74	61
9.	300	100	300	100	75	67
			% of nutrient output			
			N			
1.	—	—	—	100	154	207
6.	150	50	150	100	109	121
9.	300	100	300	100	98	106
			P_2O_5			
1.	—	—	—	100	137	172
6.	150	50	150	100	115	121
9.	300	100	300	100	102	108
			K_2O			
1.	—	—	—	100	130	155
6.	150	50	150	100	100	111
9.	300	100	300	100	95	99
			Ca			
1.	—	—	—	100	142	197
6.	150	50	150	100	114	120
9.	300	100	300	100	97	110
			Mg			
1.	—	—	—	100	154	219
6.	150	50	150	100	107	113
9.	300	100	300	100	97	103

the yield of the control grew, the yield surplus, on the other hand, was moderate (56–87% and 35–65%, respectively).

With 300 kg/ha N applied the yield of the grass mixture and those of the mixed grasslands of various legume ratio where nearly on the same level, since under the influence of fertilization large the same plant stands and plant compositions with (the legumes driven out) developed during the 4 years of the experiment.

Plant height. The tendency showed by the yearly totalled cm height of plants was similar to that of the yield.

Components. The values confirm the larger quantities of N, P, Ca and Mg in grasslands with higher ratios of legumes (Table 2). At the N 300 level, with the legumes driven out, the composition parameters are nearly identical in the various mixtures.

Comparison of the grass mixture and the grasslands of different legume ratio. Table 3. shows the yield-, dry matter- and nutrient production percentages of grasslands with various legume ratios compared to the grass mixture.

Without fertilization the various legume content grasslands produced considerable yield surpluses of dry matter (32 and 58%) and nutritive elements (30–119%, respectively).

With increasing rates of fertilization the results gradually were levelled up, because the effect of legumes ceased.

According to the data of the nutrient balance the results of the grass mixture were more favourable as regards the utilization of fertilizers (Table 4.). At the same time in the control the grassland of 18% legume ratio fixed 57 kg/ha, the one of 32% legume ratio 114 kg/ha N form the atmosphere. In the N 150 treatment the corresponding data were: 22 and 54 kg/ha, respectively, because of a decrease in the number of leguminous plants. With 300 kg/ha N applied the differences in N output became minimum (owing to the absence of legumes), and from the atmosphere nitrogen was not practically used.

Table 4

Percentage of fertilizer utilization by a grass mixture and grasslands with various ratios of legumes (1982–1985)

Number	Treatment			Grass mixture			18%			32%		
	N	P_2O_5	K_2O				ratio of legumes					
	kg/ha			N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O
6.	150	50	150	102	73	146	78	69	110	62	52	111
9.	300	100	300	86	59	114	64	48	88	55	41	80
				Total								
6.	150	50	150		117			91			82	
9.	300	100	300		94			72			64	

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OPTIMUM TIME OF REST AND N-NUTRITION OF GRASSLAND SECTIONS

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In the grazing season the seasonally different yields of grasslands can be influenced by maintaining resting periods and supplying nitrogen. In the experiment the optimum treatment whereby the yields of the grassland sections were to be made more uniform was determined for early, medium and late beginning of grazing. In spring a 20-, in summer a 30- and in autumn a 40-day rest with the use of 60 kg/ha N was found to be the most efficient. On the average of the experiment the time of rest determined the yield in 28- and the rate of N fertilization in 72 per cent.

Keywords: Fertilization, grassland, grazing, time of rest (resting period)

Introduction

In order to make the periodically changing yields of grasslands more uniform the time of rest and the quantity of N supply should be optimized for the different sections of grassland. Klapp (1952) found out that the seasonal yield fluctuation of the grassland can be lessened by the proper distribution of nitrogen. To balance the periodical differences of grass yields. Zürn, (1953) kept various — short, medium and long — resting periods which varied with the season. Voisin (1968) found that the different resting periods influenced the yield.

Baskay Tóth (1962) called attention to the monthly varying yield proportions of grasslands and to the consequent seasonal changes in their capacity to support animals. Nagy (1964, 1976) ensured the balance of grass yield and grass requirement in the grazing season by changing the time of rest and the grazing area both in the case of sectional grazing and that based on operative units. He elaborated a system of N distribution for dry- and irrigated grasslands and for their successive growth, respectively (Nagy, 1977).

Materials and methods

Between 1972 and 1974 in the district of Hortobágy-Szásztelek we studied by simulated grazing the effects of 20, 30 and 40 days of rest and 0, 60, 120, 180 kg/ha N per rotation on an irrigated natural *Poa pratensis* var. *angustifolia* — *Alopecurus pratensis* grassland given a basic

Table 1

Treatments in the experiment

Number of treatment	Time of rest day	Time of grazing and N-distribution, dose of N, month, decade														
		April 1–2	May			June			July			August			September		
			1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1		0	L0		L0		L0		L0		L0		L0		L0		L
2		1	L1		L1		L1		L1		L1		L1		L1		L
3		2	L2		L2		L2		L2		L2		L2		L2		L
4		3	L3		L3		L3		L3		L3		L3		L3		L
5		0		L0		L0		L0		L0		L0		L0		L	
6	20	1		L1		L1		L1		L1		L1		L1		L	
7		2		L2		L2		L2		L2		L2		L2		L	
8		3		L3		L3		L3		L3		L3		L3		L	
9		0			L0		L0		L0		L0		L0		L0		L
10		1			L1		L1		L1		L1		L1		L1		L
11		2			L2		L2		L2		L2		L2		L2		L
12		3			L3		L3		L3		L3		L3		L3		L
13		0	L0			L0			L0			L0			L0		L
14		1	L1			L1			L1			L1			L1		L
15		2	L2			L2			L2			L2			L2		L
16		3	L3			L3			L3			L3			L3		L
17		0		L0			L0			L0			L0			L	
18	30	1		L1			L1			L1			L1			L	
19		2		L2			L2			L2			L2			L	
20		3		L3			L3			L3			L3			L	
21		0			L0			L0			L0			L0			L
22		1			L1			L1			L1			L1			L
23		2			L2			L2			L2			L2			L
24		3			L3			L3			L3			L3			L
25		0	L0				L0				L0				L0		L
26		1	L1				L1				L1				L1		L
27		2	L2				L2				L2				L2		L
28		3	L3				L3				L3				L3		L
29		0		L0				L0				L0				L	
30		1		L1				L1				L1				L	
31	40	2		L2				L2				L2				L	
32		3		L3				L3				L3				L	
33		0			L0				L0				L0				L
34		1			L1				L1				L1				L
35		2			L2				L2				L2				L
36		3			L3				L3				L3				L

L = grazing (simulated) yield determined on the last day of the decade
0 = N 0 kg/ha
1 = N 60 kg/ha
2 = N 120 kg/ha
3 = N 180 kg/ha

fertilization of 120–210 kg PK/ha. The experiment was set up with four replications, in random block design, on plots of 24 m^2 each, in a so-called after 10, 20 and 31 May, from the end of the 1st, 2nd and 3rd decade, initiating early, medium and late grazing, so as to produce the results of the resting periods and the N-doses separately for almost every 10 days in the 10 May — 30 September period of the grazing season (Table 1). With the start of the first decade 5, 6 and 8

Table 2

Distribution of N-doses with various periods of rest on irrigated Poa pratensis var. angustifolia — Alopecurus pratensis grazing land: yields of the 1972–1974 experiment
(Year-level evaluation)

Number of treatment	Time of rest day	Number of		N kg/ha		Dry matter yield t/ha	Average of rotations t/ha	Yield per day kg/ha	Yield per day and 1 kg N kg · 10^{-3}
		rotation	decade	per rotation	total				
1		8	1	—	—	4.56	0.57	28.5	—
2		8	1	60	480	7.90	0.99	49.4	103
3		8	1	120	960	8.65	1.08	54.1	56
4		8	1	180	1440	8.93	1.12	55.8	39
5		7	2	—	—	4.34	0.62	31.0	—
6	20	7	2	60	420	7.54	1.08	53.9	128
7		7	2	120	840	7.99	1.14	57.1	68
8		7	2	180	1260	7.99	1.14	57.1	45
9		7	3	—	—	3.88	0.55	23.7	—
10		7	3	60	420	6.46	0.92	46.1	110
11		7	3	120	840	6.93	0.99	49.5	59
12		7	3	180	1260	7.18	1.03	51.3	41
13		6	1	—	—	4.15	0.69	23.1	—
14		6	1	60	360	7.38	1.23	41.0	114
15		6	1	120	720	8.57	1.43	47.6	66
16		6	1	180	1080	8.14	1.36	45.2	42
17		5	2	—	—	4.16	0.83	27.7	—
18	30	5	2	60	300	7.27	1.45	48.5	162
19		5	2	120	600	8.34	1.67	55.6	93
20		5	2	180	900	8.51	1.70	56.7	63
21		5	3	—	—	3.90	0.78	26.0	—
22		5	3	60	300	6.71	1.34	44.7	149
23		5	3	120	600	7.82	1.56	52.1	87
24		5	3	180	900	7.79	1.56	51.9	58
25		5	1	—	—	4.97	0.99	24.9	—
26		5	1	60	300	8.06	1.61	40.3	134
27		5	1	120	600	9.38	1.88	46.9	78
28		5	1	180	900	9.92	1.98	49.6	55
29		4	2	—	—	3.97	0.99	24.8	—
30	40	4	2	60	240	7.08	1.77	44.3	185
31		4	2	120	480	8.86	2.22	55.4	115
32		4	2	180	720	9.44	2.36	59.0	82
33		4	3	—	—	4.04	1.01	25.3	—
34		4	3	60	240	6.15	1.54	38.4	160
35		4	3	120	480	7.63	1.91	47.7	99
36		4	3	180	720	7.86	1.97	49.1	68
$LSD_{5\%}$						0.85			

rotations were obtained, while with the others one less. On the basis of the number of rotations and the quantities of N/rotation, very large annual quantities of nitrogen were examined: 480–960—1440 kg/ha with the 20-day, 360–720–1080 kg with the 30 day and 300–600–900 kg/ha N with the 40 day rotation in the rotation started in the first decade, and the N-dose of one rotation less in the others (Table 2).

The area of the experiment was flooded on three occasions a year, in each case with about 100 mm water (i.e. about 300 mm a year) applied by trickling irrigation.

The N-fertilizer was distributed before the rotations started, the P- and K-fertilizers were supplied once in autumn. The N-fertilizer war 34% ammonium-nitrate, the P-fertilizer 18% granulated superphosphate, while potassium was applied in the form of 40% KCl. The soil of the experiment was a medium meadow solonetz; the results of soil analysis of the 0—20 cm layer before the experiment was set up were: pH (KCl) 5.5, K_A 39, total salt % 0.04, humus % 4.1, Al-soluble P_2O_5 24 and K_2O 274 mg/kg.

The amounts of precipitation in the experimental years were 411, 420 and 614 mm, the annual mean temperatures 10.1, 9.5 and 10.0 °C, respectively (the 50-year averages are: 583 mm and 10.0 °C).

The results were evaluated by variance — and multifactorial regression analysis.

Results

Annual grass yield

The yearly evaluation of yield (Table 2) proved that there were yield differences between rotations started in different decades; usually the result of the earlier rotation was more favourable. The rotation with 40 days of rest starting after the 1st decade of May gave the best series of data for the N-doses examined. The average yield of rotations was the highest with the 40-day, the yield per day with the 20-day period of rest.

The correlations were evaluated also by multivariate regression analysis. In the bifactorial analysis the yields of grazing rotations started from different decades were evaluated separately too, so after the 1-3 decades the regression equation, the R, and the percentage effects of the time of rest and quantity of N on the basis of the partial determination coefficients:

$y_1 = 3.11 + 0.079$ time of rest $+ 0.004$ N-dose, $\quad R = 0.86$, 29% and 71%
$y_2 = 2.91 + 0.074$ time of rest $+ 0.004$ N-dose, $\quad R = 0.84$, 27% and 73%
$y_3 = 2.84 + 0.062$ time of rest $+ 0.003$ N-dose, $\quad R = 0.85$, 28% and 72%

In the trifactorial correlation examination the effects of decades were also analysed, so on the average of the experiment:
$Y = 3.83 + 0.071$ time of rest $- 0.423$ decade $+ 0.004$ N-dose, $R = 0.86$
where 24% is the time of rest, 11% the effect of the decade and 65% the effect of the quantity of N on the yield.

Yields of rotations

Even more important in the experiment were the yield level and — distribution of the different resting periods and *N*-doses during the grazing season. The yields of rotations following the 1st, 2nd and 3rd decade of May are shown in Table 3 and Fig. 1. The most uniform distribution of yield was ensured by the 30-day rest.

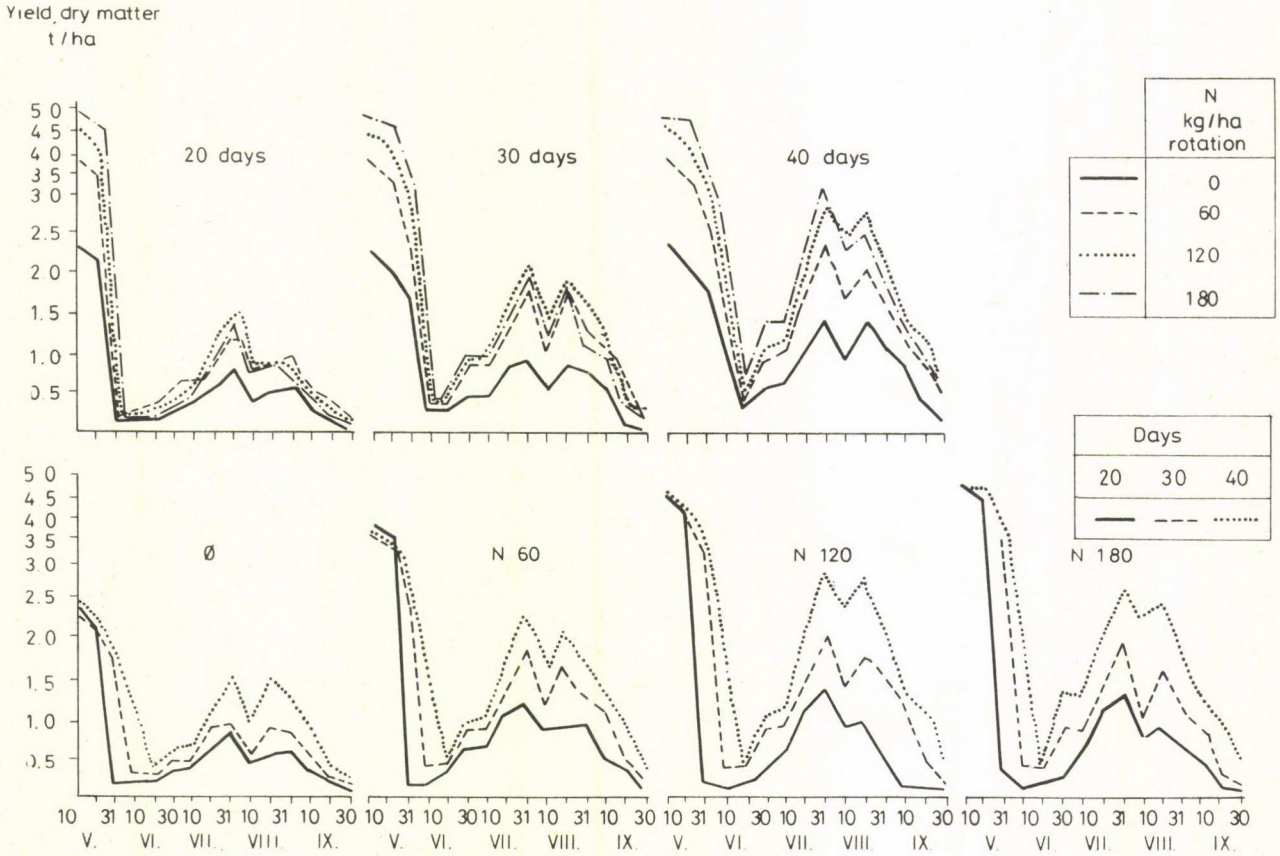

Fig. 1. N distribution for irrigated grassland with different periods of rest

The table may serve as a basis of estimation, since the data show the time of rest and quantity of N reasonably chosen for the section of grassland to be grazed at the given time so as to satisfy a group of animals of given number and grass requirement. Further information appears in Table 4, which contains the grass yields per day; on this basis we give the optimum treatment, the time of rest and quantity of N for each 10-day period.

Table 3

Yields of rotations every 10 days during the grazing season on an irrigated Poa pratensis var. angustifolia — Alopecurus pratensis grazing land, with various N-doses and periods of rest in 1972–1974 (Cumulative data)

Time of rest day	N kg/ha	Dry matter yield kg/ha in the rotations														
		May			June			July			August			September		
		10	20	31	10	20	30	10	20	31	10	20	31	10	20	30
20	—	2320	2160	100	120	140	280	390	590	790	480	520	590	280	120	20
	60	3920	3530	190	160	280	620	680	1010	1220	890	960	980	560	350	90
	120	4570	4160	200	190	210	400	640	1120	1460	920	960	1000	530	200	80
	180	4940	4730	300	140	180	360	610	1110	1350	790	970	720	510	140	70
30	—	2280	2090	1710	250	250	410	470	790	950	560	860	790	570	170	40
	60	3820	3450	2540	370	410	820	830	1270	1860	1170	1610	1300	1100	530	190
	120	4530	4140	3140	430	450	890	910	1450	2010	1410	1780	1590	1210	520	220
	180	4820	4730	3540	410	340	970	860	1470	1920	1060	1660	1190	920	310	170
40	—	2350	2200	1770	—	310	530	680	—	1460	930	1410	—	830	310	180
	60	3870	3460	2650	—	470	970	1050	—	2340	1669	2040	—	1290	990	410
	120	4580	4230	3160	—	520	1050	1170	—	2790	2410	2770	—	1410	1170	530
	180	4840	4870	3570	—	490	1310	1320	—	3190	2260	2440	—	1330	1000	530

From here: rotation results, before: set-up of the decades

At the beginning of grazing shorter time of rest and larger N-dose, in June the 30-day, and at the end of the grazing season the 40-day rotation gave a higher daily yield.

Efficiency

Yields and yield surpluses per day and kg N are shown in Table 5 and 6 and Fig. 2. On this basis we established the optimum treatments for the different periods. As regards the time of rest, at the beginning of the grazing season 20 days, in the middle 30 and at the end 40 days are optimum. The most efficient quantity of N was 60 kg/ha per rotation with each time of rest.

In general the above treatments considered efficient should be used, though occasionally larger N-doses and a longer time of rest may be necessary to ensure sufficient yield on a given section of grassland. In some cases it is more important to ensure grazing than to maintain the efficiency of a section.

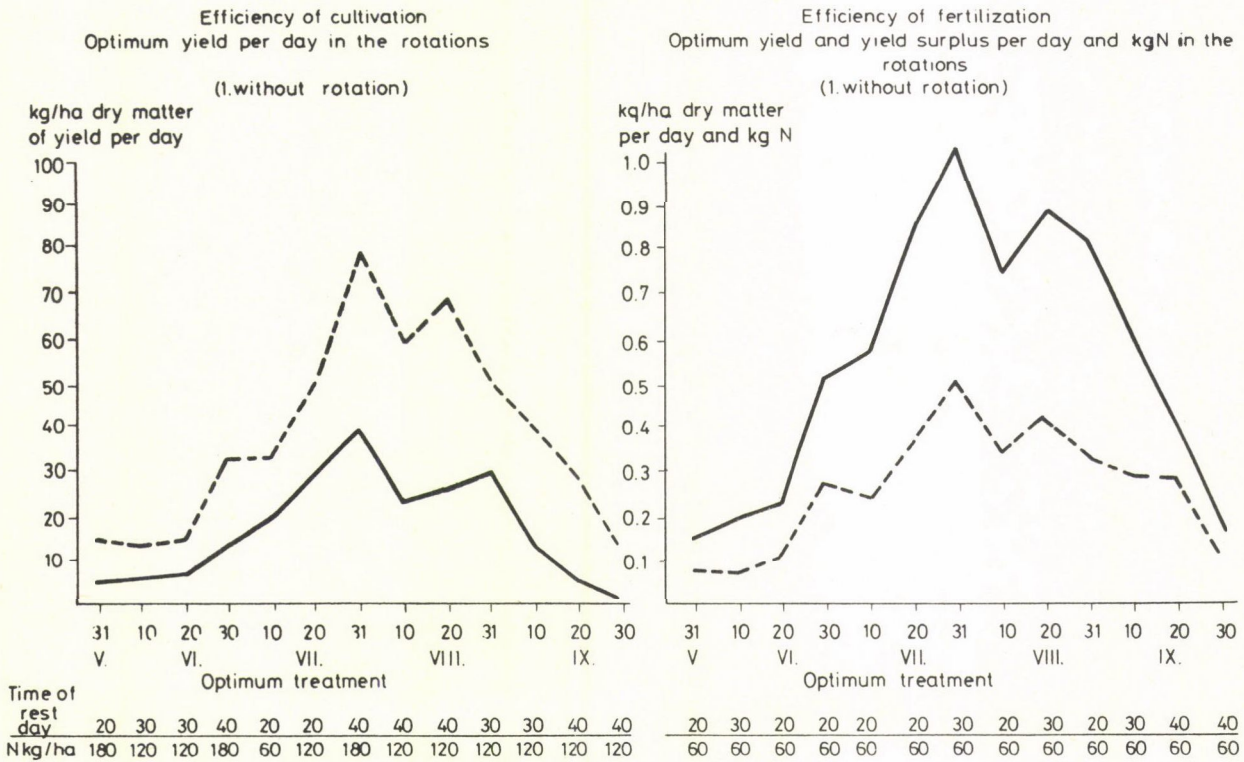
Efficiency of cultivation
Optimum yield per day in the rotations
(1.without rotation)
kg/ha dry matter of yield per day
100
90
80
70
60
50
40
30
20
10
31 10 20 30 10 20 31 10 20 31 10 20 30
V. VI. VII. VIII. IX.
Optimum treatment
Time of rest day 20 30 30 40 20 20 40 40 40 30 30 40 40
N kg/ha 180 120 120 180 60 120 180 120 120 120 120 120 120
Efficiency of fertilization
Optimum yield and yield surplus per day and kgN in the rotations
(1.without rotation)
kg/ha dry matter per day and kg N
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
31 10 20 30 10 20 31 10 20 31 10 20 30
V VI. VII. VIII. IX.
Optimum treatment
20 30 20 20 20 20 30 20 30 20 30 40 40
60 60 60 60 60 60 60 60 60 60 60 60 60

Fig. 2.

Table 4

Yield per day in the rotations in given 10-day intervals during the grazing season on an irrigated Poa pratensis var. angustifolia — Alopecurus pratensis grazing land, 1972–1974

Time of rest day	N kg/ha	Dry matter yield, kg/ha in the rotations														
		May			June			July			August			September		
		10	20	31	10	20	30	10	20	31	10	20	31	10	20	30
20	—	116	108	5	6	7	14	20	30	40	24	26	30	14	6	1
	60	196	177	10	8	14	31	34	51	61	45	48	49	28	18	5
	120	229	208	10	10	11	20	32	56	73	46	48	50	27	10	4
	180	247	237	15	7	9	18	31	56	68	40	49	36	26	7	4
30	—	76	70	57	8	8	14	16	26	32	19	29	26	19	6	1
	60	127	115	85	12	14	27	28	42	62	39	54	43	37	18	6
	120	151	138	104	14	15	30	30	48	67	47	59	53	40	17	7
	180	161	158	118	14	11	32	29	49	64	35	55	40	31	10	6
40	—	59	55	44	—	8	13	17	—	37	23	35	—	21	8	5
	60	97	87	66	—	12	24	26	—	59	42	51	—	32	25	10
	120	115	106	79	—	13	26	29	—	70	60	69	—	35	29	13
	180	121	122	89	—	12	33	33	—	80	57	61	—	33	25	13
Optimum treatment																
Time of rest		20	20	30	30	30	40	20	20	40	40	40	30	30	40	40
N kg/ha		180	180	180	120	120	180	60	120	180	120	120	120	120	120	120

On the basis of the yield data, with the different resting periods and N quantities the periodical animal supporting capacity of the grassland section can be calculated, and on the basis of the grass yields of rotations the area of grassland required to meet the fodder demand of grazing can be planned.

Summary

Between 1972 and 1974 on an irrigated natural *Poa pratensis* var. *angustifolia* — *Alopecurus pratensis* grassland in the district of Hortobágy-Szásztelek we examined the effect of 20, 30 and 40 days of rest and 0, 60, 120 and 120 kg/ha N on the different grassland sections given a basic fertilization of PK 120–210 kg/ha. Further rotations, the simulated grazing cycles imitating early, medium and late grazing started from the end of the 1st, 2nd and 3rd decade of May. The soil of the experiment was a medium meadow solonetz.

As regards the annual yield, the rotation with 40 days of rest starting from the end of the 1st decade of may gave the best results with the quantities of N examined. The yield average of the rotations was the highest in the case of 40 days of rest, while the yield per day with the 20-day resting period. According to the correlation the yields of the rotations starting from different decades between 10 and 31 May were determined in 27–29% by the time of rest, and in 71–73% by the quantity of N supplied. With the effects of decades also taken into account, on the average of the experiment the yield was determined in 24% by the time of rest, in 11% by the decade and in 65% by the N-dose.

On the basis of the yields of the grazing rotations the optimum time of rest and quantity of N can be chosen for a given grassland section and time, whereby the necessary amount of grass can be ensured. At the beginning of the grazing season the shorter, in summer the medium and in autumn the longer period of rest are optimum; while, as regards efficiency, 60 kg/ha is the optimum quantity of N. On the basis of the data of the experiment the area required for grazing can be planned and the animal supporting capacity calculated.

Table 5

Yield per day and 1 kg N in the rotations in given 10-day intervals during the grazing season on an irrigated Poa pratensis var. angustifolia — Alopecurus pratensis grazing land (1972—1974)

Time of rest day	N kg/ha	Dry matter yield per day and 1 kg N in the rotations, kg/ha														
		May			June			July			August			September		
		10	20	31	10	20	30	10	20	31	10	20	31	10	20	30
20	60	3.27	2.94	0.16	0.13	0.23	0.52	0.57	0.84	1.02	0.74	0.80	0.82	0.47	0.29	0.08
	120	1.90	1.73	0.08	0.08	0.09	0.17	0.27	0.47	0.61	0.38	0.40	0.42	0.22	0.08	0.03
	180	1.37	1.31	0.08	0.04	0.05	0.10	0.17	0.31	0.38	0.22	0.27	0.20	0.14	0.04	0.02
30	60	2.12	1.92	1.41	0.21	0.23	0.46	0.46	0.71	1.03	0.65	0.89	0.72	0.61	0.29	0.11
	120	1.26	1.15	0.86	0.12	0.13	0.25	0.25	0.40	0.56	0.39	0.49	0.44	0.34	0.14	0.06
	180	0.89	0.88	0.66	0.08	0.06	0.18	0.16	0.27	0.36	0.20	0.31	0.22	0.17	0.06	0.03
40	60	1.61	1.44	1.10	—	0.20	0.40	0.44	—	0.98	0.69	0.85	—	0.54	0.41	0.17
	120	0.95	0.88	0.66	—	0.11	0.22	0.24	—	0.58	0.50	0.58	—	0.29	0.24	0.11
	180	0.67	0.68	0.50	—	0.07	0.18	0.18	—	0.44	0.31	0.34	—	0.18	0.14	0.07
Optimum treatment																
Time of rest		20	20	20	30	20	20	20	20	30	20	30	20	30	40	40
N kg/ha		60	60	60	60	60	60	60	60	60	60	60	60	60	60	60

Table 6

Yield surplus per day and 1 kg N in the rotations in given 10-day intervals during the grazing season on an irrigated Poa pratensis var. angustifolia — Alopecurus pratensis grazing land, 1972–1974

Time of rest day	N kg/ha	Dry matter yield surplus, kg/ha, per day and 1 kg N														
		May			June			July			August			September		
		10	20	31	10	20	30	10	20	31	10	20	31	10	20	30
20	60	1.33	1.14	0.08	0.03	0.12	0.28	0.24	0.35	0.36	0.34	0.37	0.33	0.23	0.19	0.06
	120	0.94	0.83	0.04	0.03	0.03	0.05	0.10	0.22	0.28	0.18	0.18	0.17	0.10	0.03	0.03
	180	0.73	0.71	0.06	0.01	0.01	0.02	0.06	0.14	0.16	0.09	0.13	0.04	0.06	0.01	0.01
30	60	0.86	0.76	0.46	0.07	0.09	0.23	0.20	0.27	0.51	0.34	0.42	0.28	0.29	0.20	0.08
	120	0.63	0.57	0.39	0.05	0.06	0.13	0.12	0.18	0.29	0.24	0.26	0.22	0.18	0.10	0.05
	180	0.47	0.29	0.34	0.03	0.02	0.10	0.07	0.13	0.18	0.09	0.15	0.07	0.06	0.03	0.02
40	60	0.63	0.53	0.39	—	0.07	0.18	0.15	—	0.37	0.30	0.26	—	0.19	0.28	0.10
	120	0.46	0.42	0.29	—	0.04	0.11	0.10	—	0.28	0.31	0.38	—	0.12	0.18	0.07
	180	0.35	0.37	0.25	—	0.03	0.11	0.09	—	0.24	0.18	0.14	—	0.07	0.10	0.05
Optimum treatment Time of rest		20	20	20	30	20	20	20	20	30	20	30	20	30	40	40
N kg/ha		60	60	60	60	60	60	60	60	60	60	60	60	60	60	60

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NITROGEN FORMS IN PLANTS AS AFFECTED BY NITROGEN SOURCE

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The comparative effect of seven nitrogen sources, namely, ammonium chloride, ammonium nitrate, ammonium sulphate, calcium nitrate, urea, urea nitrate, and glutamic acid, applied at the levels 0, 10, and 15 meq N/litre of culture solution, was studied on N form of broad bean, maize, safflower, and sunflower plants at 240 and 480 hrs. after transplanting the emerged seedlings.

Application of nitrate-nitrogen sources enhanced the NO_3—N accumulation in the plants. Nitrate nitrogen contents were extremely low, almost negligible. Ammonium nitrogen showed direct correlation with N concentration in the medium; broad bean possessed the highest figures, followed by sunflower, maize, and safflower respectively. Ammonium sulphate followed by urea were highest in NH_4—N accumulation in all plants, whilst calcium nitrate was lowest.

Contents of organic N were raised by either raising the N level or advancing the seedlings' age; broad bean attained the uppermost figures. Maize and sunflower exchanged the second and the third position according to the N source and level, whereas safflower occupied the last position. Urea and ammonium sulphate for both broad bean and maize, and urea for the two oil-seed-plants induced the best results. Glutamic acid and ammonium cloride gave the least values for all plants tested.

Keywords: Nitrogen forms, nitrogen in plants (broad bean, maize, safflower, or sunflower), nitrogen sources, N-nutrition, N-uptake (recovery or absorption)

Introduction

Nitrogen is central to the growth and development of all plants and required in large amounts. The kind of plants, the N supply in relation to plant needs, and the N carrier are factors affecting response to an increment of N (Viets, 1965). Succeeding increments of N fertilizer produce successively larger growth rate of non-legumes (Steenbjerg, 1954; Boawn et al. 1963). Growth of legumes has been reported to be enhanced by N fertilization, especially at early stages (Eaglesham et al., 1983; Yamanka and Holl, 1984).

Most plant roots are thought to absorb nitrate more rapidly than ammonium (Novoa and Loomis, 1981), although there are reports (Berlier and Guiraud, 1967; Spratt and Grasser, 1970) that young seedlings prefer ammonium. Plant species differ in their response to different sources of N nutrition (Kirkby, 1981).

The present work was designed in order to make a comparison betwen seven sources of nitrogen, applied at varying levels to culture solution, in affecting the content of nitrogen forms in broad bean, maize, safflower, and sunflower plant seedlings, at two young ages.

Materials and methods

Seeds (or kernels) of broad bean (*Vicia faba* L.), maize (*Zea mays* L.), safflower (*Carthamus tinctorius* L.), and sunflower (*Helianthus annuus* L.) were soaked in aerated distilled water for 48 hrs to germinate, then transferred into a cloth floating over 0.25×10^{-2} M $CaCl_2$ solution and left to emerge for four more days in the dark (Handly et al. 1960). Four seedlings of each plant were transplanted onto plastic disc (1.5 mm thick) having 4 holes, floating over one litre of nutrient solution contained in glass cylinder. Long Ashton's nutrient solution was prepared according to Hewitt and Smith (1975). Nitrogen level in the culture medium was 0, 10, or 15 meq N/L from each of seven sources, namely ammonium chloride, ammonium nitrate, ammonium sulphate, calcium nitrate, urea, urea nitrate, or glutamic acid.

The experiment made use of four replicates. Maize and sunflower were planted in May (late spring), and broad bean and safflower in November (late autumn), according to their natural growing seasons. Experimental temperatures were the room abient ones, i.e. 24–28 °C in late spring and 18–22 °C in late autumn. Nutrient solution, with the nitrogen source applied, was kept throughout at pH 6.0 by adding either NaOH or HCl as required. Elements concentration and sterility were maintained at almost a constant by periodical changes of the nutrient solution. The culture medium was aerated, by pump, for two hours daily.

Plant samples were collected at 240 and 480 hrs. after transplanting the seedlings (to the culture solution), dried at 70 °C and finely ground, Nitrogen forms in the dried plants were determined according to the methods given by Horwitz (1970), namely, total nitrogen-by distillation of the wet acid digest, using semi-micro *Kjeldahl* technique; mineral nitrogen-by reduction to ammonia, in the $CaCl_2$—CCl_3COOH plant extract, using ferrous sulphate and granular zinc, then distillation in alkaline medium by micro-steam distillation, in alkaline medium, nitrite nitrogen — colourimetrically, using *Gries-Ilosvay* reagents; nitrate nitrogen — by subtracting data of the ammoniacal and nitrite nitrogen from those of the mineral nitrogen; organic nitrogen by calculating the difference between the total nitrogen and the mineral one.

Results

Nitrate nitrogen

Application of the nitrogen sources, particularly the nitrite ones, resulted generally in augmenting nitrate content in the biomass (Fig. 1). Updosing the nitrogen added led to high increases in NO_3—N content in the plants; such increases were greatly pronounced in road beans. Rate of nitrate accumulation in the other plants tested followed the order: sunflower > maize > safflower.

The nitrogen sources applied, in this study, almost showed the same order for the four plants tested concerning nitrate content of the seedling tissues. Such order was: calcium nitrate > ammonium nitrate > urea nitrate. The sources providing merely reduced nitrogen, generally, showed no notable changes in NO_3—N content of the plants, although, very slight increases were obtained in few cases. Baker and Maynard (1971) reported that nitrogen source, rate, method and time of application, and environmental conditions, notably temperature, might influence nitrate accumulation.

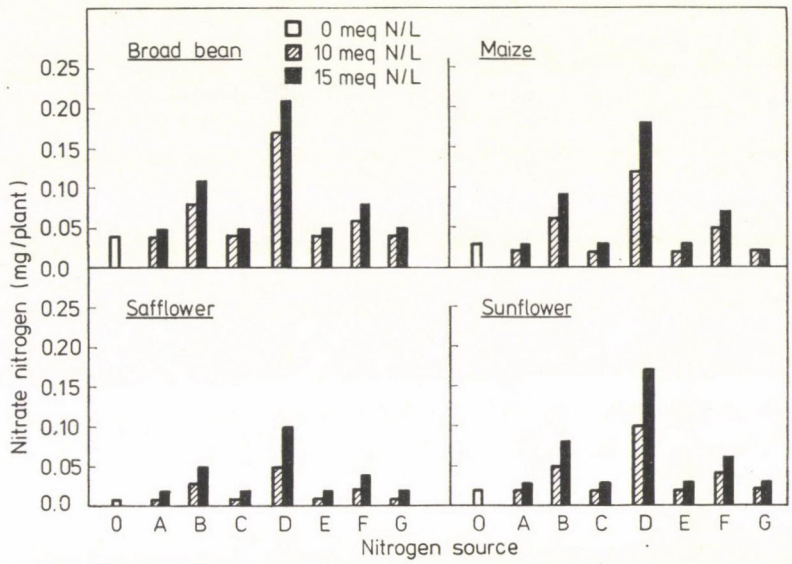

Fig. 1. Nitrate nitrogen in the plants treated with different sources of nitrogen, at 240 hrs. after transplanting. (O = Control; A = Ammonium chloride; B = Ammonium nitrate, C = = Ammonium sulphate, D = Calcium nitrate; E = Urea; F = Urea nitrate; G = Glutamic acid)

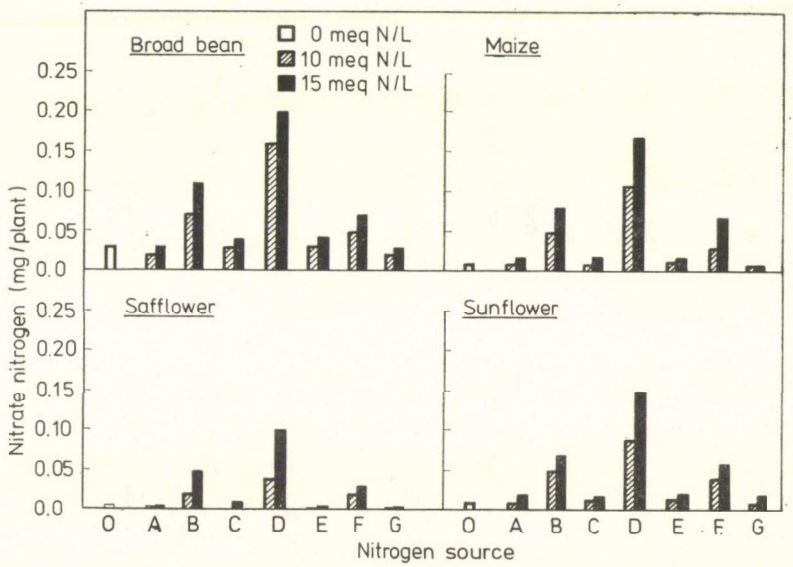

Fig. 2. Nitrate nitrogen in the plants treated with different sources of nitrogen, at 480 hrs. after transplanting. (0 = Control; A = Ammonium chloride; B = Ammonium nitrate, C = = Ammonium sulphate; D = Calcium nitrate; E = Urea, F = Urea nitrate; G = Glutamic acid)

Aging of seedlings, in the present work, had implied upleveling of NO_3—N in the plants supplied with nitrate N sources (Fig. 2). The other non-nitrate sources of nitrogen had revealed fluctuating values with time. No alterations had taken place regarding the above-mentioned orders for both the plants and N sources, although the control values had diminished with time, due to utilization of the initial seed-stored-NO_3-N in protein construction required for the growing plants.

Nitrite nitrogen

NO_2-N contents were, in general, extremely low, even absent in many variables. Introduction of nitrate nitrogen sources relatively surpassed the other sources as to give detectable NO_2-N amount in the plant tissues. No NO_2-N was absolutely observed in safflower for all treatments at both sampling times.

Ammonium nitrogen

It was generally shown that NH_4^+-N content in the plants examined was directly correlated with the nitrogen concentration in the culture solution. Broad bean possessed, relatively, the highest NH_4^+-N contents, for all nitrogen

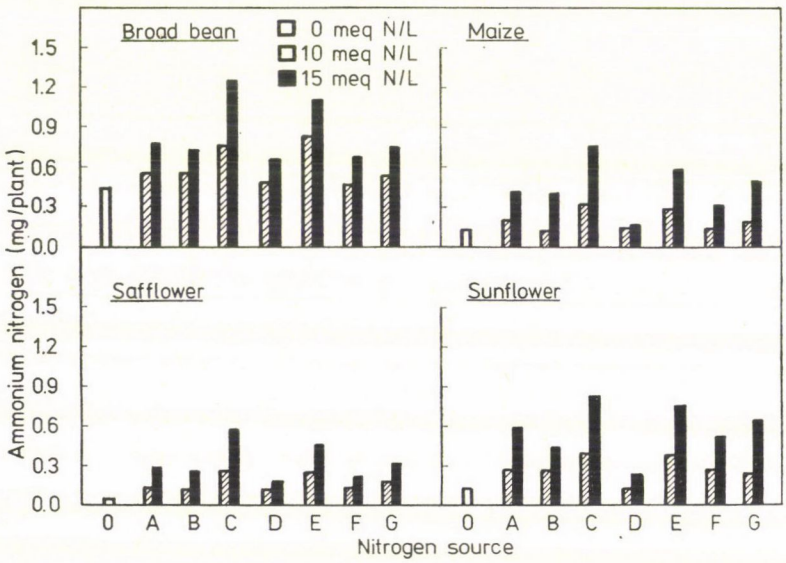

Fig. 3. Ammonium nitrogen in the plants treated with different sources of nitrogen, at 240 hrs. after transplanting. (0 = Control; A = Ammonium chloride; B = Ammonium nitrate, C = = Ammonium sulphate, D = Calcium nitrate; E = Urea, F = Urea nitrate, G = Glutamic acid)

sources applied, and followed by sunflower, maize, and safflower respectively (Fig. 3).

The four plants, of this study, denoted analogy in their response to the various nitrogen sources used. Ammonium sulphate followed by urea then glutamic acid and ammonium chloride showed, respectively, the highest contents of NH_4^+-N in the plants, whereas calcium nitrate was of the least values; urea nitrate and ammonium nitrate revealed intermediate effects.

Fluctuations in HN_4^+-N contents had generally occurred by advancing the plants'age (Fig. 4), although the values tended to diminish in most cases. This is ascribed to the active NH_4^+ consumption to face the promoting accumulation rate of photosynthetic materials as plants grow up. Williams (1955) noted that ammonium did not accumulate in the plant until the plant was injured by excessive NH_4^+ nutrition.

Organic nitrogen

The content of organic nitrogen in the plants studied progressively increased with increasing the nitrogen level applied from the different sources, as well as by advancing plant age as vegetative growth proceeded (Figs 5 and 6). Nature of the plant governed the accumulation extent of organic nitrogen n the biomass, namely, the legume broad bean maintained the top content,

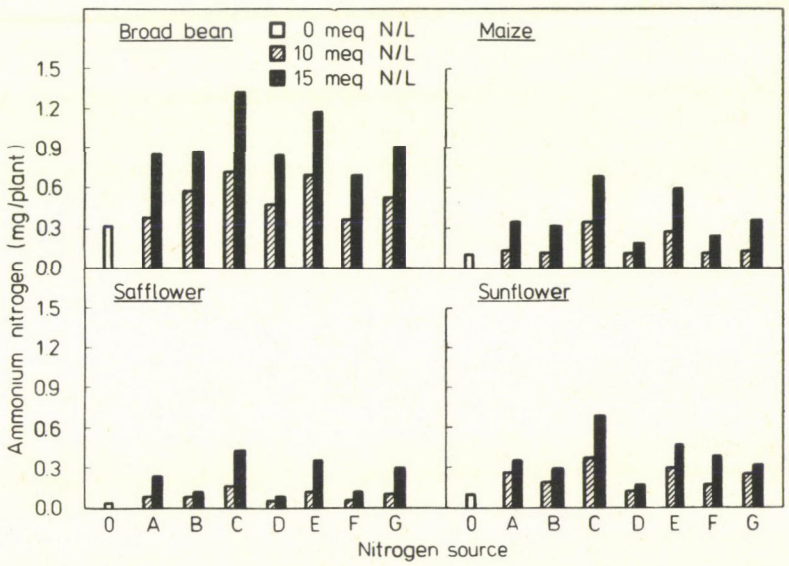

Fig. 4. Ammonium nitrogen in the plants treated with different sources of nitrogen, at 480 hrs. after transplanting. (0 = Control; A = Ammonium chloride; B = Ammonium nitrate, C = = Ammonium sulphate, D = Calcium nitrate; E = Urea, F = Urea nitrate; G = Glutamic acid)

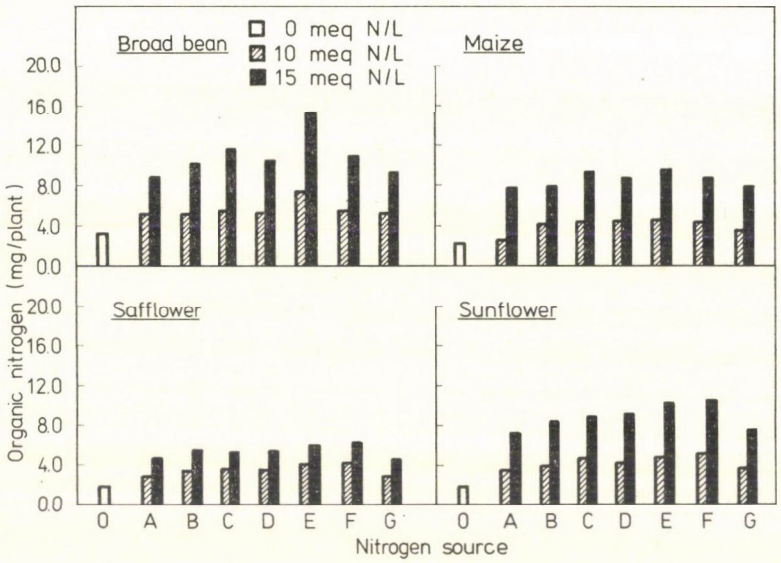

Fig. 5. Organic nitrogen in the plants treated with different sources of nitrogen, at 240 hrs. after transplanting. (0 = Control; A = Ammonium chloride, B = Ammonium nitrate, C = = Ammonium sulphate, D = Calcium nitrate, E = Urea, F = Urea nitrate, G = Glutamic acid)

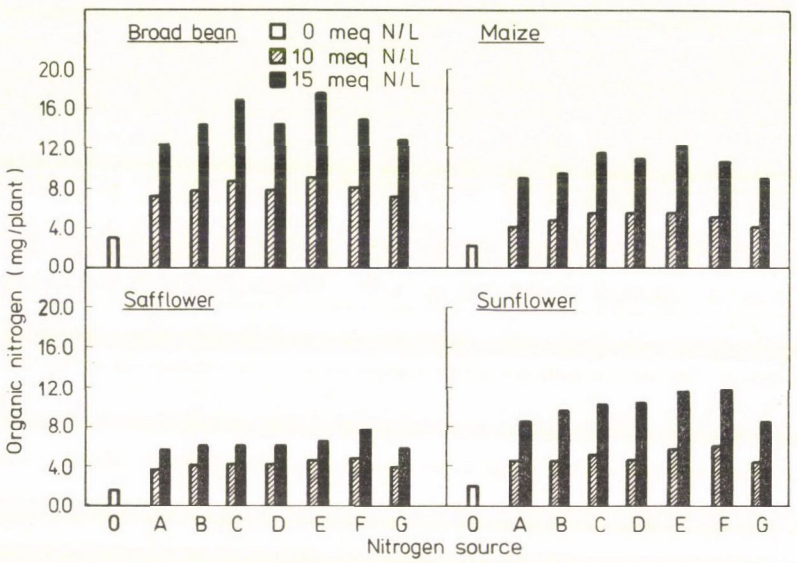

Fig. 6. Organic nitrogen in the plants treated with different sources of nitrogen, at 480 hrs. after transplanting. (0 = Control; A = Ammonium chloride, B = Ammonium nitrate, C = = Ammonium sulphate, D = Calcium nitrate, E = Urea, F = Urea nitrate, G = Glutamic acid)

followed by the cereal maize and the oil-seed-sunflower (both are almost convergent), then the oil-seed-safflower respectively. This is controlled by genotype of the plant (Huffaker and Rains, 1978). The control plants suffering nitrogen deficiency showed minor changes, tending to show very slight increases, by sampling time. Such minute increases arose from the conversion of the seed-stored-nitrate and ammonium nitrogen. Stahlin (1967) pointed out that the content of organic N compounds was raised with increasing doses of fertilizer N; as the dry-matter content of the plant tissue tended to decrease rather than increase with higher doses of N, the increase in organic N compounds resulted in a decrease of other compounds, mainly carbohydrates. Schrader et al. (1972) found that contents of organic N in maize plants increased (from 990 to 1724 mg) as the ammonium concentration in the nutrient solutions was increased from 50 to 150 ppm; however, assimilation of nitrate was maximal at 100 ppm (980 mg) and actually decreased slightly at 150 ppm (933 mg); since less organic N accumulated in the nitrate treatments and growth was somewhat slower.

Urea, urea nitrate, and ammonium sulphate implied the highest figures in all plants tested, while glutamic acid and ammonium chloride were the least. Omran et al. (1979) working on the fertilization of horse bean and barley, using area, ammonium sulphate, and ammonium nitrate, found that urea gave the greatest total N content in horse bean, whilst ammonium sulphate surpassed the other sources in such concern for barley; ammonium nitrate had the lowest figures for horse bean and intermediate ones for barely.

Discussion

Application of nitrate nitrogen sources led to rising the nitrate content in the plants tested, overriding the other nitrogen sources. Combination of nitrate with other nitrogen sources, e.g., ammonium nitrate and urea nitrate lessened the NO_3 content of the plants, comparing with calcium nitrate. This is due, logically, to the initial relative content of NO_3-N in the compound used. Baker and Maynard (1971) found that nitrate concentrations in spinach were highest when KNO_3 served as the nitrogen source and were less when NH_4NO_3 and urea was the nitrogen must be supplied as nitrate to avoid ammonium toxicity. Schrader et al., (1972) pointed out that uptake of NO_3 was not retarded by presence of NH_4 but assimilation of NO_3 into organic N was retarded by NH_4^+. Huffaker and Rains (1978) reported that calcium accelerated the absorption of nitrate by plants. They interpreted this in the way that the positively charged calcium masks the negative charges of the cell wall, this allows the anions to migrate more closely to the plasma membrane uptake sites. They also added that ammonium ions have an inhibitory effect on nitrate

uptake; the effect seems to be on the transport system, in some way directly inhibiting the transport of nitrate.

In some instances, nitrate or nitrite is formed from ammonium within plant tissues (Hewitt and Smith, 1975). Such oxidation process may be mediated by peroxidase, catalase, or methaemoglobin. The production of nitrate, nitrite, or nitro-compounds by such means may explain the occurrence of activity of nitrate reductase in circumstances where the enzyme, although inducible, appears under conditions which should have excluded exogenous supplies of nitrate or nitrite. This could help to interpret the slight rise in NO_3-N occurred in some cases of the plants supplied with non-nitrate-nitrogen sources in the present study.

Urea is hydrolyzed, as it enters plant roots, to ammonia by means of the enzyme urease. Such process is rapid and needs no energy expenditure by the plant. Hence introduction of ammonium nitrogen, as well as urea as sources of nitrogen to plants growing on culture solution led, as expected, to higher NH_4^+-N content in the plant tissues. Ammoniacal nitrogen appearing in the plants treated with all nitrogen sources represented the transite amount in the way to amino acids build-up. Nitrate nitrogen, when absorbed, must be firstly reduced to ammonia prior to incorporation into organic molecules, whilst ammonium nitrogen can be directly incorporated.

Roots absorb urea faster than either NH_3. Hirose and Goto (1961) found that urea entered roots of rice in solution culture and could be detected throughout the plant. They could detect no urease activity in the roots. Urea uptake did not suppress K^+ uptake as NH_4^+ did. They concluded that urea enters the root by simple diffusion, whereas NH_4^+ competes for the same carrier complex as K^+. Such finding supports ours regarding the superiority of urea in most cases.

It had been reported that high rates of nitrogen are required for immediate plant requirements especially at youthful stages. The young plants store the excess nitrogen, which is translocated within the tissues when needed later in the growth period (Pumphery and Harris, 1956). Plant type likewise, determines the kind of roots which in turn influences the absorption capacity, i.e., deep tap roots are more efficient in N intake than surface fibrous roots (Huffakes and Rains, 1978).

The present results led to the conclusion, that, urea, urea nitrate, and ammonium sulphate showed the best nitrogen recovery by the plants tested. The propable toxicity of chloride anion in ammonium chloride, as well as the high molecular weight of glutamic acid might be behind their least figures. Ammonium combined with nitrate in ammonium nitrate did not prove to be satisfactory for nitrogen uptake by the plants. This means that the source of nitrogen determined the N form and recovery in plant tissues rather than the kind of plant itself.

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FERTILIZATION OF GRASSES AND MIXED GRASSLANDS

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Fertilization significantly increased the yield of all grasses and of the mixed grassland. According to the results of the experiment under the given conditions (soil, climate, etc.) a 5-year long-term action of fertilization did not cause essential differences between the pure stands of the grasses examined and the mixed grassland either in yield or in nutrient output.

In the yield of the control the differences of grasses are decisive. The nutrient content of the soil was best utilized by the blue grass and the dactylis.

In fertilizer utilization only the tall fescue exceeded the mixed grassland.

With higher levels of N, equalization occurred. The NPK output of high yielding grasses of lower nutritive value was nearly the same as that of the mixed grassland and grasses of higher value of components yielding somewhat less.

The use of a grass mixture or one or another grass species should be decided on the basis of the purpose of cultivation and utilization.

Keywords: fertilization, components, grasses, mixed grassland, yield

Introduction

Using grasses in pure stand has recently become a wide practice owing to their large yields and excellent reactions to N-fertilization.

The mixed grasslands no longer profit from very high rates of N-fertilization, and changes occur in the composition of plants: grasses responsive to N become dominant and the legumes are repressed.

Beyond the volume of yield the quality of the crop was also subjected to examination in order to decide whether it was to a mixed grassland or to a pure stand of some grass that preference had to be given when planting a new grassland. We examined therefore the production potential, the way of making use of the natural nutrient supply of the soil, the reactions to fertilization, the utilization of fertilizers, the specific values of components and the nutrient output.

Ecker (1983) attaches importance to planting single-species grasslands in order to ensure the continuity of grazing and lengthen the grazing season.

Nagy (1978) examined over several years the various irrigated grasses and mixed grasslands used by grazing and cutting, respectively. In the case of cutting the order of value was: grass mixture, tall fescue, dactylis, meadow fescue, dactylis and tall fescue.

Many researchers studied various grasses associated with a legume: in the case of lower rates of N used the associations gave higher yields and nutritive values, and at the same time ensured savings of fertilizers compared to the grasses; while larger quantities of N resulted in a levelling up (In: Nagy 1978).

Materials and methods

Between 1974 and 1978 fertilization experiments were carried out in Debrecen on a chernozem soil with 5 grasses and a grass mixture. The grasses were: Hungarian brome grass ("Szarvasi-52"), tall fescue ("Szarvasi-56"), meadow fescue ("Szarvasi-54"), blue grass ("Szarvasi-36") and dactylis ("Szarvasi-51"). The blue grass was replanted every second year.

The composition of the grass mixture was: meadow fescue 14-, tall fescue 6-, dactylis 5-, red fescue ("G") 4-, blue grass ("Szarvasi-59") 3-, timoty ("G") 2-, white clover ("Lovászpatonai") 4- and bird's foot trefoil ("G") 2 kg/ha.

The data of analysing the 0–20 cm layer of the soil before the experiment was set up were: pH (KCl) 7.3; K_A 40; total salt % 0; $CaCO_3$% 1.6; humus % 1.6; P_2O_5 56; K_2O 36 mg/kg.

In the years of the experiment precipitation and mean temperature were about the same as their 50-year average (583 mm, 10.0 °C).

The treatments of the experiment are contained in Table 1.

The laboratory analyses were performed by an ICP apparatus with the NAK methods of the Ministry of Agriculture and Food; the calculations were made with a R-10 computer.

Results and discussion

The experiment was evaluated on the basis of the 5-year average results.

Yield

The fertilizer treatments resulted in significant differences for all plants (Table 1). At the different N-levels the grasses showed a smaller or greater variation of yield. In the control the monocultures — except the Hungarian

Table 1

Yield of grasses and a grass mixture in response to fertilization
(1974–1978)

Number	Treatment			Dry matter production t/ha											
	N	P_2O_5	K_2O	Grass mixture		Hungarian brome grass		Tall fescue		Meadow fescue		Blue grass		Dactylis	
	kg/ha				%		%		%		%		%		%
1	—	—	—	2.00	100	1.17	100	2.33	100	3.01	100	4.42	100	3.21	100
2	120	60	80	4.98	249	3.61	308	6.77	290	6.25	207	7.58	172	6.89	214
3	240	80	120	9.05	452	7.10	607	10.06	431	8.23	272	11.14	252	9.83	306
4	360	100	160	11.07	553	9.02	771	11.94	512	10.23	339	12.42	281	11.39	355
$LSD_{5\%}$				1.07	54	1.09	93	1.42	61	1.29	43	1.23	27	1.19	37

brome grass — produced 17–121 per cent higher yields compared to the grass mixture. The natural nutrient supplies of the soil were best utilized by the blue grass and dactylis. At the 120 and 240 levels of N the differences lessened. With 360 kg/ha N the blue grass produced 12% and the tall fescue 8 per cent more than the grass mixture; the meadow fescue and the Hungarian brome grass fell behind with their yields (see Table 5).

With the yield surplus the situation was different. In the N 120 treatment several grass monocultures still exceeded the mixed grassland, but at the 240 and 360 levels of N only the tall fescue did so. To higher rates of N-fertilization the meadow fescue was the least responsive.

Components

As a result of the fertilizer treatments the N-, P-, K-, Mg-, Zn and Cu-content increased, and the quantities of Ca and Mn decreased (Table 2).

Table 2

Components of fertilized grass monocultures and a mixed grassland
(% of dry matter in) 1974–1978

Component	Number of treatment	Grass mixture		Hungarian brome grass		Tall fescue		Meadow fescue		Blue grass		Dactylis	
			%		%		%		%		%		%
N %	1	1.62	100	1.60	100	1.36	100	1.55	100	1.32	100	1.41	100
	3	1.77	109	1.88	118	1.54	113	1.77	114	1.42	108	1.51	107
	4	1.96	121	2.11	132	1.89	137	2.01	130	1.59	120	1.75	124
P %	1	0.23	100	0.26	100	0.24	100	0.31	100	0.25	100	0.25	100
	3	0.24	104	0.27	104	0.25	100	0.32	103	0.28	112	0.26	104
	4	0.25	109	0.28	108	0.25	104	0.34	110	0.30	120	0.27	108
K %	1	1.77	100	2.48	100	2.14	100	2.29	100	2.13	100	2.55	100
	3	2.17	123	2.76	111	2.47	115	3.00	131	2.33	109	2.70	106
	4	2.47	139	2.98	120	2.56	120	3.32	145	2.58	121	2.86	112
Ca %	1	0.52	100	0.45	100	0.36	100	0.35	100	0.52	100	0.33	100
	3	0.44	85	0.41	91	0.34	97	0.31	97	0.48	92	0.31	94
	4	0.41	79	0.39	87	0.34	94	0.33	94	0.44	85	0.30	91
Mg %	1	0.22	100	0.19	100	0.23	100	0.20	100	0.18	100	0.21	100
	3	0.25	114	0.21	111	0.24	104	0.23	115	0.18	106	0.24	114
	4	0.28	127	0.22	116	0.26	113	0.25	125	0.19	112	0.25	119

For the different nutritive elements the values of the grasses and grass mixture examined as well as their order of rank differed. Their evaluation by specific nutritive element content is:

N%: in the control it was the highest in the mixed grassland, but in the N 360 treatment the Hungarian brome grass and the meadow fescue exceeded the grass mixture in N concentration.

P%: the grass mixture was exceeded by all grass species in P concentration, with higher rates of N-fertilization in an increased measure. The meadow fescue and the blue grass excelled in high P-content. In response to fertilization a 4–12% surplus was obtained in the N 240- and 4–20% in the N 360 treatment

K%: The grass monocultures attained in all treatments a higher K level compared to the mixed grassland. The K-content was highest in the meadow fescue, Hungarian brome grass and dactylis stands. In the N 240 treatment 6–31%, while in the case of N 360 12–45% surplus K was produced by the grass species examined.

Ca%: In the grasses the Ca content was lower than in the grass mixture, except the blue grass. Increasing rates of N-fertilization reduced the Ca content, in the N 360 treatment by 9–21%.

Mg%: In the grass mixture the Mg content was slightly higher than in the grasses. As a result of N-fertilization the Mg content increased, in the N 360 treatment by 12–27%.

Nutrient output, fertilizer utilization

The NPK output and fertilizer utilization of the grasses are shown in Table 3.

In N utilization the mixed grassland and the tall fescue monoculture were the best with 51–53% compared to the 38–48% of the other grasses.

In P utilization the blue grass and the meadow fescue (57–60%) exceeded the other grasses.

In K utilization the result was above 100% for all stands owing to the available K-content of the soil. The meadow- and the tall fescue were the best of all (193–204%).

In total NPK utilization the grass mixture was exceeded by the tall fescue while the other grasses sowed more or less the same values.

Economic efficiency

We evaluated the effect of fertilization (Table 4) on the total yield and the yield surplus, and calculated the quantities of total- and N active ingredient used in the treatments to produce 1 t dry matter output. The order of grasses changes with the doses.

For total yield the blue grass, for yield surplus the tall fescue, had the most favourable result. The diversity was due to differences in production potential and nutrient utilization under the given ecological conditions.

Table 3

Nutrient output of grasses and a grass mixture, and percentage of fertilizer utilization (1974–1978)

Number of treatment	Nutrient output kg/ha (A) and fertilizer utilization % (B)											
	Grass mixture		Hungarian brome grass		Tall fescue		Meadow fescue		Blue grass		Dactylis	
	A	B	A	B	A	B	A	B	A	B	A	B
					N output							
1	32.4	—	18.7	—	31.7	—	46.7	—	58.3	—	45.3	—
3	160.2	53	133.5	48	154.9	51	145.7	51	158.2	42	148.4	43
4	217.0	51	190.3	48	222.1	53	205.6	44	196.2	38	199.3	43
					P_2O_5 output							
1	10.6	—	7.0	—	12.6	—	21.1	—	25.6	—	18.6	—
3	49.8	49	44.0	46	55.3	53	60.1	49	71.3	57	59.0	51
4	62.0	51	58.6	52	66.9	54	79.8	59	85.7	60	70.6	52
					K_2O output							
1	42.6	—	35.0	—	60.1	—	83.1	—	113.6	—	98.5	—
3	237.1	162	235.7	167	298.9	199	297.9	179	313.0	166	319.5	184
4	328.8	179	323.8	181	368.9	193	409.2	204	386.3	170	393.0	184
					Total nutrient output							
1	85.9	—	60.7	—	104.4	—	150.9	—	197.5	—	162.4	—
3	447.1	82	413.2	80	509.0	92	503.7	80	542.5	78	526.9	83
4	607.8	84	527.7	83	657.9	89	69.46	88	668.2	76	662.9	81

Table 4

Efficiency of fertilization to grass monocultures and a mixed grassland (1974–1978)

Number	Treatment			Total and N active ingredient used for 1 t dry matter output											
	N	P_2O_5	K_2O	Grass mixture		Hungarian brome		Tall fescue		Meadow fescue		Blue grass		Dactylis	
	kg/ha			total	N	total	N	total	N	total	N	total	N	total	N
							Total yield								
2	120	60	80	52.2	24.1	72.0	33.2	38.4	17.7	41.6	19.2	34.3	15.8	37.7	17.4
3	240	80	120	48.6	26.6	62.0	33.9	43.8	23.9	53.5	29.2	39.5	21.6	44.8	24.5
4	360	100	160	56.0	32.6	68.8	40.0	51.9	30.2	60.6	35.2	49.9	29.0	54.5	31.7
							Yield surplus								
2	120	60	80	87.2	40.2	106.6	49.1	58.6	27.0	80.4	37.1	82.3	37.9	70.6	32.5
3	240	80	120	62.4	34.1	74.2	40.5	57.0	31.1	84.4	46.1	65.5	35.8	66.5	36.3
4	360	100	160	68.3	39.7	79.0	45.9	64.5	37.5	86.0	50.0	77.5	45.1	75.9	44.1

Comparison of the grasses and the grass mixture

The results of the fertilization treatments are compared in Table 5 on the basis of the major parameters. In the control the grass species showed a decisive variation of total yield, because the nutrient supplies of the soil were best utilized by the blue grass and the dactylis. In responsiveness to fertilization the tall fescue and the dactylis excelled. In total NPK utilization only the tall fescue exceeded the grass mixture.

Table 5

Fertilization results of grass monocultures and of a mixed grassland
1974–1978
(% in proportion to the grass mixture)

Number	N	Treatment P_2O_5 kg/ha	K_2O	Grass mixture	Hungarian brome grass	Tall fescue	Meadow fescue	Blue grass	Dactylis
				Total yield %					
1	—	—	—	100	59	117	151	221	161
2	120	60	80	100	72	135	126	152	138
3	240	80	120	100	78	111	91	123	109
4	360	100	160	100	81	108	92	112	103
				Yield surplus %					
2	120	30	80	100	82	149	109	106	123
3	240	80	120	100	84	110	74	95	94
4	360	100	160	100	87	106	80	88	90
				Total nutrient output %					
1	—	—	—	100	71	122	176	231	190
3	240	80	120	100	71	122	176	231	190
4	360	100	160	100	94	108	114	110	109
				Fertilizer utilization %					
3	240	80	120	100	99	110	99	93	99
4	360	100	160	100	99	106	105	90	96

In conclusion it can be established that in the fertilizer treatments — especially in the N 240 — and N 360 ones — no essential difference was found between the mixed grassland and the various grass monocultures. The use of pure stands of grasses should be determined by the purpose of cultivation and utilization: they may be of importance first of all in hay-fields, in the case of making hay or silage, when the uniformity of the plant stand has a role.

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Plant genetics and breeding

SOMACLONAL VARIATION IN THE R_3-GENERATION OF A MAIZE INBRED LINE

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Immature embryos of the maize line CHI-31 were cultured on N-6 basal medium containing 2.5 uM 2,4-D and 2470 plantlets regenerated from somatic embryos. The phenotypic variation of 554 somaclones was screened in the R_2-generation and progenies were tested in the R_3-generation for confirmation of the selected traits. Genetic variability for some quantitative traits of agronomic interest was assessed in the R_3-generation. Progeny analysis of somaclones confirmed that heritable somaclonal variation was generated. Eight sublines exerted significant differences in plant height and flowering date to the parent maize inbred line CHI-31. The study aimed at identifying the nature and extent of somaclonal variation and substantiating its genetic potential for maize improvement.

Keywords: *in vitro* culture, *iv vitro* plant regeneration, quantitative genetic variability, somatic embryogenesis, *Zea mays*

Introduction

Somaclonal variation, a novel source of genetic variability, has been subject to many investigations and interpretations (for recent review see Larkin, 1987). Green and Phillips (1975) were among the first reporting on *in vitro* regeneration of maize from immature embryos. Lu et al. (1982) and Novak et al. (1983) provided evidence that plant regeneration in maize was achieved through somatic embryogenesis.

Plant regeneration was though earlier to be restricted to a few maize inbred lines. However, Lu et al. (1983) studied the regeneration capacity of eleven maize F_1-hybrids and observed a rapid proliferation of the scutellum and formation of embryogenic callus on 2,4-D containing media. Embryoids of these hybrids gave rise to normal green plants. There is evidence, however, that processes of somatic embryogenesis and plant regeneration in maize tissue culture strongly depend on the genotype of the donor plant (Nesticky et al., 1983; Beckert and Quing, 1984, Tomes and Smith, 1985, Novak et al.,

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1988). *In vitro* regenerated maize plants and their selfed progenies were reported to express a varying degree of phenotypical variation (Edallo et al., 1981, Beckert et al. 1983; Goebel et al. 1986; Zehr et al. 1987; Novak et al. 1986a, b 1988). This variation derived from *in vitro* systems has been termed "somaclonal variation". It seems to be a frequent phenomenon in maize tissue culture. The nature of somaclonal variation in plant tissue culture is still not well understood. Our work concentrated on the evaluation of variability in the breeding programme of maize.

Materials and methods

This investigation is part of a set of experiments in which *in vitro* derived somaclonal variation was assessed and compared to radiation induced variability (Novak et al., 1986a, b; 1988).

The maize inbred line CHI-31 was chosen for its high *in vitro* regeneration ability (Novak et al., 1983). Immature caryopses were harvested 8–12 days after selfing, sterilized for 30 minutes in 20% Chlorox (TM) solution and rinsed three times with sterile water. Approximately 1–1.2 mm long immature embryos were excised from the kernels and aseptically transferred to test tubes with the scutellum side upwards and the plumule-radicle in contact with the N-6 nutrient culture medium (Chu et al., 1975) containing 2.5 μM 2,4-D (2,4-dichlorophenoxyacetic acid) and 120 g/l sucrose.

Cultures were incubated at 25 °C and 16 hours-light photoperiod (2000 lux). The subculture intervals were 20 days. After 120 days of culture on the same medium, plant regeneration was initiated upon transfer of the embryogenic callus to hormone free medium with 60 g/l sucrose.

R_1 -plantlets derived from tissue culture were aseptically transplanted into perlite saturated with half strength nutrient solution and cultured during 14 days in a growth chamber and then transplanted into soil mixed with peat and perlite.

R_1-plants were transplanted to the field and selfed whenever possible but frequent protandric or protogynic behaviour necessitated in many cases backcrosses with the CHI-31 inbred parent. This procedure delayed selection for one generation.

Selfed R_1-plants were harvested separately and plant progenies screened in the R_2-generation for phenotypic variation. Variants were then progeny tested with 25 seeds of each progeny for confirmation of the selected traits in the R_3-generation.

Plant heights wre measured twice at early vegetative development and at harvest. Silking dates were recorded and the dry matter weight per plant assessed after harvest.

Results and discussion

Chlorophyll deficiencies

The regenerated R_1-plants exhibited a large phenotypic variation in leaf colour pigmentation and in plant morphology. Virescent or pale green seedlings and plants with white or yellow stripe leaf variegation were the most frequent changes. Albina and lutescens plantlets died after transplantation to perlite. Though most of the pale green maize seedlings survived in the field and produced seeds, the pale green seedling colour could not be confirmed in the R_2-generation. Yellow or white leaf variegations were scored in 63 plants or in 7% of the total R_1-generation. Variegation usually occurred in small longi-

tudinal leaf sectors of plants derived from the same embryogenic callus, indicating that somaclonal differences exist also within regenerated plants. The sectorial manifestation of morphological changes supports this conclusion. Different chlorophyll and morphological deviants occurred among plants regenerated from the same explant. These phenotypical differences were found also among multiple platlets growing from a single embryogenic clump and in the segregating progenies of the R_3-generation (Novak et al., 1988). This phenomenon suggests a mutational sectoring of callus culture (Zehr et al., 1987.).

Most plants with variegated leaves developed no kernels. The growth was considerably delayed and flower formation irregular which led to protandric and protogynic plants. While R_2-progenies of 11 chlorophyll variegated plants turned to normal, variegation was confirmed in 3 lines only; their plant progenies were further tested and the variability of some quantitative traits assessed (Table 1). Since all plants within and among the 3 progenies were

Table 1

Flowering dates and dry matter yield in R_3-maize somaclones from the inbred line CHI-31. The same letters indicate non-significant differences between lines

Line	No. of plants	Silking date in August Mean ± s.e.	Dry matter yield per plant, g Mean ± s.e.	No. of plants with seeds
CHI-31, Orig. line	41	6.6 ± 0.4 b	165.1 ± 3.9 a	41
30-2, Variegated	15	9.1 ± 0.7 c	100.2 ± 9.6 f	3
30-3, Variegated	16	11.8 ± 0.6 def	79.7 ± 7.3 g	5
30-4, Variegated	20	13.1 ± 0.6 efg	98.7 ± 7.5 f	4
21-2, Short	22	15.7 ± 0.4 h	114.9 ± 5.1 e	22
22-3, Short	20	14.8 ± 0.6 gh	123.9 ± 2.3 d	18
75-1, Short	19	13.2 ± 0.4 fg	121.7 ± 3.8 d	18
126-1, Short	18	10.9 ± 0.6 cde	125.9 ± 3.8 cd	18
174-2, Short	17	9.8 ± 0.4 cd	129.2 ± 3.4 c	16
184-3, Short	17	9.0 ± 0.3 c	131.5 ± 4.1 c	16
130-2, Early	21	2.8 ± 0.3 a	154.7 ± 3.6 b	21
161-6, Early	23	3.7 ± 0.3 a	163.5 ± 5.3 a	23

variegated throughout, their relative growth and yield depended mostly on the variegation pattern, i.e. on the relative proportion between yellow and green leaf sectors. Moreover, the average plant height of these 3 variegated lines was decreased (Figure 1) and the date of flowering delayed when compared to control plants. The variation of both analysed traits was high. Staub (1987) reported on an aberrant phenotype in maize characterized by longitudinal white leaf stripes which was attributable to and correlated with the presence of supernumerary B-chromosomes. When present in low number, B-chromosomes exert subtle influences on cell metabolism, size and mass,

but effects are not manifested phenotypically. The variegated phenotype intesifies with increasing numbers between five and thirteen B-chromosomes. A high number of B-chromosomes exhibits a quantitative influence on many generalized effects including a reduction in germnaition, growth and vigor, a changed flowering time and a decreased fertility due to an increased aborted pollen percentage (Jones, 1975).

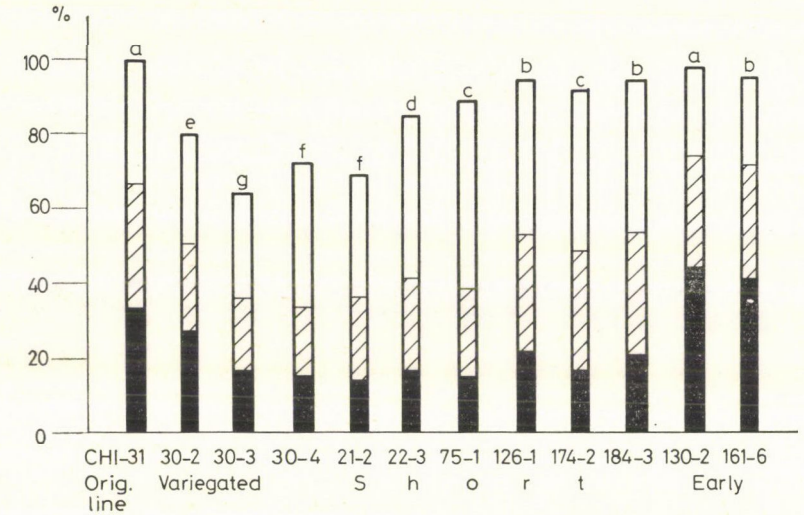

Fig. 1. Dynamics of plant growth in R_3-maize somaclones compared to the original line CHI-31. Filled bars = plant height measured on 27 June, hatched bars = plant height on 11 July and open bars = total height at maturity. The same letters indicate non-significant differences at the time of measurement. Control plant height at maturity 137.4 + 1.8 cm

These findings appear valid to explain phenomena observed and quantitatively assessed in our tissue culture derived plants with variegated leaves. A discussion on the possible origin of B-chromosomes in organisms and their influence on physiological and other generalized effects is given by Jones and Rees (1982).

Quantitative traits

Some agronomic characteristics of somaclones are shown in Table 1 and Figure 1. Their growth dynamics differed strongly during vegetative plant development: Both, plant height and date of flowering exhibited a large variation in the R_2-generation and plants showing these traits were selfed and progeny tested in the R_3-generation. A heritable variation in plant height was evident in somaclones of CHI-31 maize inbreds (Figure 2) and 6 out of 62 R_2-plants had a uniform short stature in the R_3-generation. While plant height of all 6 somaclones was significantly shorter than the one of control

plants at the first date of measurement, the relative growth rate of 3 short variants was similar between the first and second date of measurement and in the other 3 short somaclones significantly reduced as compared to the growth rate of control plants. Progenies of the regenerated lines 21-2, 22-3 and 75-1 differed significantly in plant height to the control plants at maturity. Though short stature somaclones were primarily selected for plant height, concurrent differences in the silking date and in dry matter production were observed. The other 56 short plant type R_3-progenies could not be used for an investigation of quantitative variation due to continued segregation.

Effects of a specific major gene affecting plant height could be tentatively identified in the 6 uniform short stature R_2 selections and differences in gene expression of some quantitative traits were evident in the R_3-generation. Somaclonal short variants may be conditioned by a genetic change of a

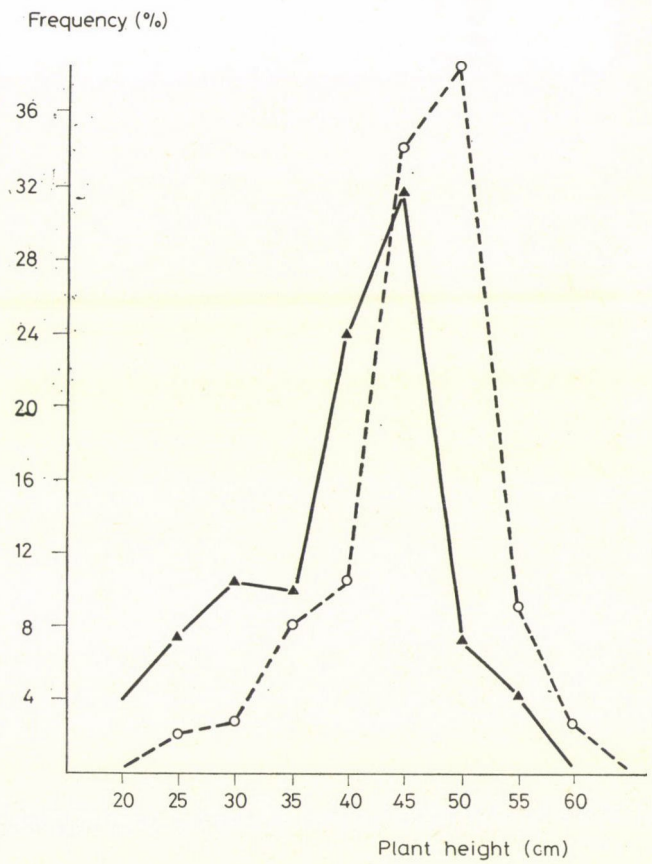

Fig. 2. Distribution of plant heights on 27 June of 172 R_2 progenies of CHI-31 maize inbred. Full line: 62 R_2 plants classified as short with 1145 progenies measured in R_3. Broken line: 113 R_2 normal height plant with 2.341 progenies in R_3

single locus which results in a dwarf genotype. Since a number of genes are known to effect and modify plant height in maize, further investigation are needed for their identification and impact on plant height within a defined genetic background.

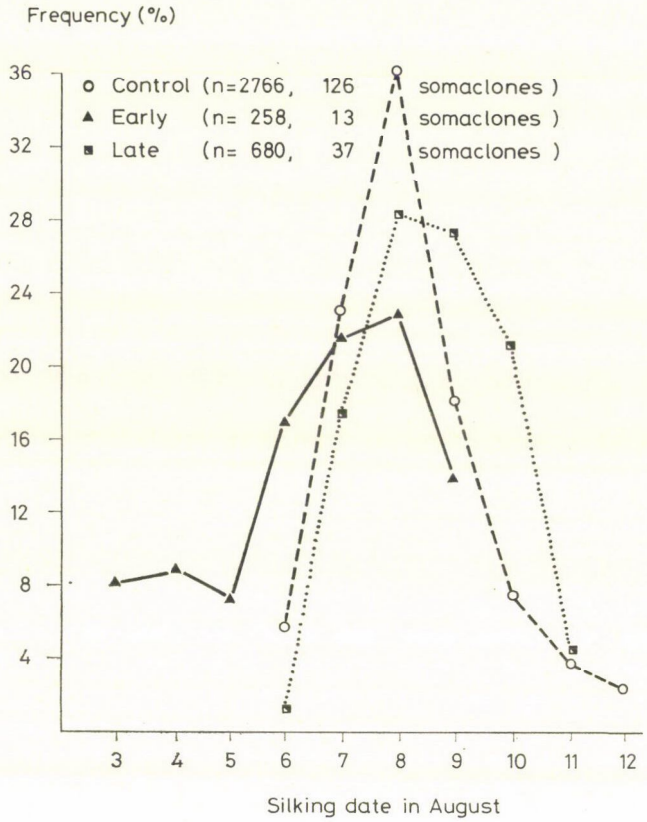

Fig. 3. Distribution in silking date of the CH-31 maize somaclones in the R_3 generation

Among the variants in flowering date of R_2-regenerants, the 13 earliest and 37 latest flowering plants were selfed and plant progenies tested quantitatively. The distribution of flowering dates (Figure 3) shows distinctly different patterns between early, control and late R_3-populations. Offsprings of the somaclones 130-2 and 161-6 were the only ones exhibiting a significantly different heritable variance in earliness (Table 1).

The graph depicted in Figure 3 is open-ended as some individuals with extreme flowering times not shown in the graph were omitted. The short plant somaclones 21-2 and 22-3 flowered 9 and 8 days later than the control population.

The results indicate that quantitative variation in plant height and flowering date was achieved through plant regeneration via somatic embryogenesis in maize. The somaclonal variation of R_2-progeny derived from CHI-31 regenerants for other quantitatively inherited subtraits of maize kernel yield, i.e. cob length, cob position on the plant, number of rows per cob and number of kernels per row, was published previously (Nesticky et al., 1984; Novak et al., 1986b).

Results obtained in a top-cross indicate that even the combining ability of the sublines derived from *in vitro* regenerants may be subject to changes which enable on application of the tissue culture system to produce sister inbred lines of maize (Beckert et al., 1983; Novak et al., 1986b). More recently, Zehr et al. (1987) observed genotypic differences in the extent of quantitative somaclonal variation and its possible application for conventional maize breeding programmes.

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EFFECT OF DIFFERENT MAIZE (*ZEA MAYS* L.) GENOTYPES ON GRAIN FODDER PRODUCTION

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Because of missing complex evaluation of different grain maize (*Zea mays* L.) genotypes, four hybrids with the same growing seasons were investigated based on the results of field and *in vivo* digestibility tests in continental climate in Hungary. The objective of this study was to provide some ideas for the choice of hybrids and for reconsidering the breeding strategy.

Significant differences were observed among the genotypes with normal germplasm in respect to grain yield, *in vivo* metabolizable energy and digestible crude protein performance. The rank orders of different genotypes according to these features differed from each other. As a matter of fact it would be concluded that some hybrids could be sufficient for high energy yield, and others for high digestible protein performance. Since grain yield was the most important factor for high metabolizable energy yield, it is possible to achieve the best properties with high yielding hybrids. Since the investigation of chemical components and the in vivo digestibility trial are expensive improvement needs a cheap and fast method. This could be the near infrared (NIR) technique.

Keywords: digestibility, energy yield, grain type, maize maturity group, protein yield

Introduction

About the fodder production of grain corn (*Zea mays* L.) our knowledge is very limited because the complex evaluation of different genotypes is neglected due to the high cost of feeding experiments.

After the finding of opaque-2 mutant (Mertz et al., 1964) every effort was made to investigate its feeding value and that of other endosperm mutants (brittle-1 and, 2, dull, soft-starch, srunken-1 and 2, sugary-1 and 2, waxy etc.). Due to their lower yield performance compared with the normal ones (Baenziger and Glover, 1979) they could not be popular in practice, so the corn with normal endosperm is, and will be the main fodder in the future. Even if protein content of soybean, barley etc. and their biological value are higher than that of grain maize, in some countries the maize has been considered as a protein-feed, due to its widespread use. In Hungary 65–70% of total fodder is corn, and it satisfies 40% of total protein-demand. Only a few papers were published on feeding values of normal corn with different genotypes. In an investigation with poultry, Leeson et al., (1977) found small differences among genotypes in metabolizable energy. Differences in the biological values of

protein in two hybrids were found by Hernandez (1981) in experiments with pigs. Some genotypes with different growing season length were investigated with pigs in Yugoslavia (Nuskern et al. 1980). In general, hybrids with longer growing seasons have proven less digestible than the earlier maturing ones. Also in Yugoslavia the different genotypes, regardless of the length of the growing season, in fattening pigs experiment produced a 599.2–647.4 g/day weight gain (Beric et al. 1981).

The different yield performance of hybrids are well known, but the complex evaluations of normal corn are missing. For this reason our main goal is to investigate (i) whether the genotypes differ in metabolizable energy and in digestible crude protein yields (ii) what would be the most important component for high energy performance and for high digestible protein yield.

Materials and methods

A three-year (1983–1985) field trial was carried out at the Research Station of the University of Mosonmagyaróvár, Hungary (north latitude 47°50' and elevation 120 m. The experimental design was split-plot with four replications. The plot size was 300 m^2; one third was harvested as grain, half of the rest used for CCM (Corn-Cob-Mix) and the rest for whole plant silage. The row spacing was 70 cm and planting was done at about 10 °C of soil temperature at 5 cm depth (last week of April or first week of May) with 8 plants/m^2 using hand-guns. The plants were harvested for grain use after black-layer formation, regardless of the length of the growing seasons of hybrids. The climatic and agronomical conditions of all three seasons allowed the non-stressed development of maize.

Four genotypes (3839, 3950, 3901 and 3906) bred by Pioneer Hi-Bred International, Des Moines, Iowa, U. S. A. were chosen. Because Nuskern (1980) found that earlier hybrids were of better quality than the later ones we determined to investigate hybrids in almost the same maturity group to detect genotype effects. In order to characterize the duration of growing season, the effective heat units (EHU) were calculated:

$$EHU = \sum_{e}^{b} \frac{T_{max} + T_{min}}{2} - 10\ °C$$

e = emergency
b = black layer formation

The harvested ear was dried at 40 °C, then shelled. The diet contained 96% corn (different genotypes according to the treatments) and 4 per cent supplement (2% AP-117*, 1% CaCO, 0.5%, NaCl and 0.5% Premix 28*). A ten-day preliminary period was applied to accustom the pigs to the diet and to single cages. During the experimental period (10 days) the daily intake of dry matter was 800 g which was applied twice daily in dry form. It was sufficient for non-stressed development.

The *in vivo* digestibility, based on the differences in chemical components of fodder and feaces, was carried out with 20 $\pm$ 2 kg barrow Hungahyb piglets, bred by the Research Center for Animal Breeding and Nutrition, Gödöllő, Hungary. The chemical analyses of both feed and faeces were done according to the Hungarian official standard (MSZ 6830), which is similar to the international (CRC) one. To avoid N-loss, the N-content of feaces was analyzed from fresh samples, and immediately after the sampling of urine toluol film was laid on the top of samples.

* prepared by Gabonatröszt, Budapest, Hungary

Results and discussion

According to the quantitative (GY) and the qualitative (GT) features (Tables 1 and 2) the investigated genotypes well represent the hybrids produced in a continental climate. Only in vivo digestibility of crude fibre is the exception. The reason for differences from Nuskern's (1980) results could be the warmer and brighter Yugoslavian climate, compared to the Hungarian. As the warmer temperature and longer photoperiod produce more lingnin in their vegetative plant part, and consequently less digestibility (Deinum and Bakker, 1981; Deinum and Struik, 1982; Struik, 1983), thus, the differences in the climate are suggested as the main reason of difference of *in vivo* crude fibre digestibility of the grain. Due to the negligible crude fibre content of grain (2.5—3.0%), this fact is of no practical importance.

Similarly to the grain yield (Table 2/GY), the performance of genotypes in metabolizable energy (MEY) and in digestible crude protein (DPY) are also

Table 1

Range of chemical features and in vivo digestibility of chemical components of investigated hybrids (A), *genotypes of* PINTÉR et al. (1985) (B) and NUSKERN et al. (1980) (C) publications

Chemical components	A		B		C	
	min.	max.	min.	max.	min.	max.
	Chemical features (g/kg)					
Crude protein	101.2	113.7	101.1	110.1		
Crude fat	41.8	50.1	35.3	47.9		
Crude fibre	29.9	32.6	25.4	30.6		
Ashes	14.5	16.6	13.9	15.9		
N-free extracts	795.5	807.8	799.4	818.0		
	In vivo digestibility (%)					
Crude protein	75.2	79.6	77.5	79.6	72.2	84.4
Crude fat	83.5	86.1	72.4	83.1	70.2	84.3
Crude fibre	64.3	69.9	63.9	73.7	41.1	60.5
N-free extracts	90.7	92.0	91.7	93.0	92.1	93.5

different. In metabolizable energy yield the rank order of hybrids is identical to the grain performance, but in digestible crude protein yield differs from it. Consequently the MEY depends more on the GY than the DPY.

The DPY is dependent partly on GY and partly on digestible crude protein content (DCPC). The two influencing factors of DCPC were also investigated (Table 3). There were substantial differences among the genotypes in the *in vivo* protein digestibility (DP), but the crude protein content can have greater effect on the change of DCPC:

Table 2

Duration of growing season (EHU), *grain type* (GT), *grain yield* (GY) *in air dried material, metabolizable energy yield* (MEY), *digestible crude protein yield* (DPY) *of genotypes with normal endosperm*

Hybrids	EHU	GT	GY kg/m²	MEY MJ/m²	DPY g/m²
*3839	1325	sd**	1.12 c***	16.61 bc	98.31 a
*3950	1270	d	1.07 c	15.28 c	84.76 b
*3901	1329	d	1.19 b	17.29 b	89.44 b
*3906	1345	d	1.30 a	19.06 a	99.36 a

* Pioneer hybrids
** sd = semi-dent, d = dent
*** the same letter within the column indicates no significant ($P_{0.05}$) difference (Duncan, 1955)

Table 3

The grain type (GT), *crude protein content* (CPC) *and its in vivo digestibility* (DP) *of genotypes with normal endosperm*

Hybrids	GT	CPC (g/kg) (‰)	DP (%)
*3839	sd**	113.7	77.2
*3950	d	105.7	75.1
*3901	d	105.4	79.6
*3906	d	101.2	75.7
Average	—	106.5	76.9

* Pioneer hybrids
** sd = semi-dent, d = dent

When we compare the grain type to the protein features, the DP could be independent from the grain shape. At the same time the CPC was higher with semi-dent than with dents. It corresponded to earlier experiences noticed at F2 and F4 progenies, but we had to emphasize, according to our earlier findings (Pintér et al., 1985) that this statement was not characteristic for every semi-dent, but only for F2 and F4 progenies.

In conclusion we found significant differences among the genotypes both in metabolizable energy yield and in digestible crude protein performance. Because the rank order of hybrids is not identical in energy and in protein yield, one hybrid would be more suitable for energy production (e.g. beef production) and the other for obtaining more protein (e.g. pork production).

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STUDIES ON MAIZE GENE POOLS. I. GENETIC ARCHITECTURE OF GRAIN YIELD AND OTHER AGRONOMIC TRAITS[1]

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Two gene pools of maize, namely, BC yellow pool and CD white pool, were studied under two environments during rabi 1982–83 to determine the genetic architecture of grain yield and other agronomic characters following North Carolina Design I mating system proposed by Comstock and Robinson (1948, 1952). For grain yield and its components, additive genetic variance was the major contributor to the total genetic variability in all the characters under both the populations except for kernel row per ear in CD white pool where dominance variance played a significant role. The role of G × E interaction components, additive × environment and dominance × environment was relatively unimportant. The results from the present investigation agree with the theoretical expectations that the gene pools developed from diverse selected elite materials are relatively stable and exhibit variability mostly of an additive nature.

Keywords: additive genetic variance, gene pools, G × E interaction, Zea mays

Introduction

In India a well planned intra-population improvement programme in maize was initiated in 1973. In some populations there was almost negligible gain whereas in others there was as high as 7% improvement per cycle (Gill, 1983). In view of these encouraging results and information from other sources (Gardner, 1974; Sprague and Eberhart, 1977 and Darrah et al. 1978) regarding genetic gains realized in various population improvement programmes, in order to update the breeding strategy it was resolved by the All India Coordinated Maize Improvement Project in 1979 to make renewed efforts in this direction by formulating a comprehensive programme of forming genetically broad-based gene pools and subject them to intra-population improvement.

At the initial stages of population improvement programme it is desirable to know estimates of genetic parameters which permit visualizing the potentiality of improvement through breeding. Keeping in view the importance of developing superior early maturing varieties through population improve

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ment, the present study was undertaken in two early maturing heterozygous base populations (gene pools) composed in the recent past under the All India Coordinated Maize Improvement Project.

Materials and methods

Materials for the present study consisted of two early maturing, genetically broad-based, heterozygous gene pools-BC yellow pool (henceforth referred to as P_1) and CD white pool (henceforth referred to as P_2). These pools were developed by the composition of a number of selected elite materials. A half sib nursery block consisting of 2 males and 4 females was planted for initial synthesis of each pool in Kharif-1979 followed by random mating during rabi-1979–80 JKharif-80 and rabi-1980–81. The detailed procedure of initial synthesis of these pools is given in the Proceedings of Twenty-second Workshop of All India Coordinated Maize Improvement Project, 1979.

Half-sib and full-sib progenies were developed in each of the two populations according to Robinson et al. 1948 during Kharif-1982 at the Agronomy Farm, Rajasthan College of Agriculture, Udaipur. Sixty-four male groups (A group of four progenies involving the same male parent) in P_1 and sixty male groups in P_2 were divided into 16 and 15 sets respectively. Each set comprised sixteen progenies (full-sibs) involving four male groups. Various male groups were assigned to the sets at random and the sixteen full-sib progenies within a set were also randomized. The progenies were evaluated in a randomized incomplete block design with two replications in both the populations. For each population two experiments were conducted in two different environment (henceforth referred to as E_1 and E_2) created by planting at two different dates with a time interval of one month during ragi-1982–83. In both the populations, each progeny was represented by a single row plot, 5 m long with the spacings of 0.70 m between rows and 0.20 m between plants within each row. Data on 10 competitive plants were recorded for plant height (cm), ear height (cm), kernel rows per ear (no.), days to 50% silking and grain yield per plant (gm). The mean values of each plot were subjected to statistical analyses for each environment and also pooled for both the environments in each population. The estimates of genetic components of variance, viz. additive genetic variance $\hat{\sigma}^2_A$ and dominance variance $\hat{\sigma}^2_D$ were obtained following Comstock and Robinson (1948, 1952).

Results and discussion

The estimates of components of genetic variances studied for various characters are presented in Table 1. In general, the estimates of $\hat{\sigma}^2_A$ were significant for all the characters in E_1, E_2 and pooled analysis in both the populations except that in P_1, $\hat{\sigma}^2_A$ was non-significant for kernel rows per ear in E_2 and for yield per plant in E_1 and pooled analysis. Whereas in P_2 $\hat{\sigma}^2_A$ was non-significant for kernel rows per ear in E_1 and pooled analysis and for yield per plant in E_2. The estimates of $\hat{\sigma}^2_A$ were not significant for all the characters in E_1, E_2 and pooled analysis in both the populations, except that in P_2 these were significant for ear diameter in E_1 and for kernel rows per ear in E_1 and pooled analysis.

A perusal of comparative estimates of the genetic variances would indicate that the magnitude of $\hat{\sigma}^2_A$ was much larger than that of $\hat{\sigma}^2_A$ for each character in E_1, E_2 and pooled analysis except in P_1 for ear height in E_1 and kernel rows per ear in E_2 and in P_2 for ear diameter in E_1, kernel rows per ear in E_1 and pooled analysis, and for yield per plant in E_2.

The above results thus indicated the presence of a substantial proportion of $\hat{\sigma}^2_A$ for all the characters under study in both the populations. However, in P_2, since only $\hat{\sigma}^2_A$ was significant for kernel rows per ear in E_1 and pooled data, it may be possible that $\hat{\sigma}^2_A$ contributed significantly towards the expression of total genetic variability for this character. A perusal of estimates of dominance ratio also supports the above findings, since no dominance to partial dominance level was observed in almost all the cases.

It is interesting to note that in both the populations high frequency of negative estimates was observed for $\hat{\sigma}^2_A$ Obilana et al. (1979) indicated that negative estimates could arise due to factors such as sampling variance assorta-

Table 1

Estimates of genetic components of variance and dominance ratio in P_1 *and* P_2

Character	Environment	P_1			P_2		
		$\hat{\sigma}^2_A$	$\hat{\sigma}^2_D$	$\hat{\sigma}^2_D/\hat{\sigma}^2_A$	$\hat{\sigma}^2_A$	$\hat{\sigma}^2_D$	$\hat{\sigma}^2_D/\hat{\sigma}^2_A$
Plant height	E^1	130.430**	Neg.	NE	142.300**	60.920	0.428
	E^2	644.000**	Neg.	Ne	439.200**	Neg.	NE
	Combined	231.900**	Neg.	NE	236.400**	Neg.	NE
Ear height	E^1	27.610*	36.360	1.317	31.460**	4.838	0.153
	E^2	268.300**	Neg.	NE	140.800**	Neg.	NE
	Combined	73.020*	12.290	0.168	62.950**	Neg.	NE
Leaves above the ear	E^1	0.088**	Neg.	NE	0.079**	Neg.	NE
	E^2	0.145**	Neg.	NE	0.069*	Neg.	NE
	Combined	0.091**	0.003	0.038	0.051*	0.009	0.189
Ear length	E^1	0.738*	0.136	0.174	1.806**	0.343	0.190
	E^2	1.915**	1.511	0.788	1.689**	0.539	0.319
	Combined	0.863*	Neg.	NE	1.792**	Neg.	NE
Ear diameter	E^1	0.055*	0.007	0.129	0.048*	0.082*	1.708
	E^2	0.052**	0.017	0.322	0.059*	0.013	0.227
	Combined	0.047**	0.001	0.010	0.043*	0.041	0.960
Kernel rows per ear	E^1	0.551*	0.047	0.086	0.263	1.169**	4.437
	E^2	0.161	0.524	3.256	0.694*	0.513	0.739
	Combined	0.325*	0.122	0.375	0.340	0.768**	2.257
Days to silk	E^1	67.680**	Neg.	NE	50.100**	Neg.	NE
	E^2	74.570**	Neg.	NE	38.130**	Neg.	NE
	Combined	65.260**	Neg.	NE	40.880**	Neg.	NE
Yield per plant	E^1	61.710	Neg.	NE	108.900**	Neg.	NE
	E^2	224.400**	40.120	0.178	89.410	108.900	1.218
	Combined	51.920	Neg.	NE	96.330**	Neg.	NE

Where: Neg. = The estimated were negative
NE = Could not be estimated since $\hat{\sigma}^2_D$ was negative.

tive mating, linkage effects, deficiency in the genetic model, and estimates of actual zero values. High frequency of negative estimates observed for 12 in the present study suggested that perhaps in highly broad-based populations like those used in the present study, a sample size of 256 families may not be adequate to obtain unbiased estimates of this parameter. And therefore, it is likely that a bigger sample size might help obtain estimates of greater reliability. From the foregoing results it is apparent that the gene pools under study and currently undergoing intra-population improvement exhibit a greater degree of genetic variability of additive nature for grain yield and its contributing characters. Chopra (1964), Raouto (1977) and Gill (1983) have reported high additive genetic effects in genetically broad-based populations.

The results of G×E interactions (Table 2) indicated that the main component $\hat{\sigma}^2_A$ was higher in magnitude for most of the characters in both populations than additive×environment interaction $\hat{\sigma}^2_{AI}$. However, the ratio $\hat{\sigma}^2_{DI}/\hat{\sigma}^2_D$ could not be computed in most of the cases, in both the populations, since one or the other component revealed negative estimates. The estimates of $\hat{\sigma}^2_{AI}$ were found to be significant for plant height and ear height in P_1, ear length in P_1 and P_2 and days to silk in P_1 and P_2 and non-significant for remaining characters in both the populations. Thus the results indicated that, in general, genotype×environment interactions played minor role in determining total genetic variability in both the populations. These findings are in agreement with those of Smith et al. (1978b) wherein no significant interaction across locations within each gene pool was observed. They reasoned that the gene pools were composed of a diversity of genotypes, particularly in early genera-

Table 2

Character	P_1				P_2			
	$\hat{\sigma}^2_A$	$\hat{\sigma}^2_{AI}/\hat{\sigma}^2_A$	$\hat{\sigma}^2_{DI}$	$\hat{\sigma}^2_{DI}/\hat{\sigma}^2_D$	$\hat{\sigma}^2_{AI}$	$\hat{\sigma}^2_{AI}/\hat{\sigma}^2_A$	$\hat{\sigma}^2_{DI}$	$\hat{\sigma}^2_{DI}/\hat{\sigma}^2_D$
Plant height	155.300**	0.669	Neg.	NE4	54.310*	0.229	Neg.	NE4
Ear height	74.960**	1.026	Neg.	NE3	23.190*	0.368	Neg.	NE4
Leaves above the ear.	0.025	0.274	Neg.	NE3	0.023	0.450	Neg.	NE3
Ear length	0.490	0.567	0.940	NE1	Neg.	NE2	0.940*	NE1
Ear diameter	0.10	0.212	0.010	10.000	0.010	0.232	0.010	0.243
Kernel rows per ear	0.030	0.092	0.160	1.311	0.140	0.411	0.070	0.091
Days to silk	5.860	0.089	6.380	NE1	3.240	0.079	13.310**	NE1
Yield per plant	91.15*	1.755	2.290	NE1	2.800	0.029	65.460	NE1

Where: Neg. = The estimates were negative

NE_1, NE_2, NE_3 and NE_4 indicates that the ratios could not be estimated since $\hat{\sigma}^2_D$, $\hat{\sigma}^2_{AI}$, $\hat{\sigma}^2_{DI}$, and $\hat{\sigma}^2_{DI}$ and $\hat{\sigma}^2_D$ both respectively were negative

tions, each of which could respond differently to the various environments so that the net effect would be a little interaction across locations.

The significant estimates of $\hat{\sigma}^2_A$ recorded for all the characters in both the populations suggest that there exists sufficient scope for manipulation of different characters in desirable direction through selection. Palmer and Musgrave (1966) found that photosynthate from leaves above the ear is almost exclusively translocated to ear. Tripathy et al. (1972) reported that photosynthate from upper leaves of maize is used faster in seed development than the photosynthate generated in lower leaves. Various defoliation studies (Pintér et al., 1977; Pintér 1980; Remison, 1982) have also shown that the upper leaves contribute more to grain yield in maize, and decrease in yield, due to defoliation and this varied significantly between genotypes. However, workers at Cimmyt (1977) have hypothesized that excessive leaf area above the ear in tropical maize materials may reduce the grain-producting efficiency (harvest index) of the plant. Considering these findings and predominance of $\hat{\sigma}^2_A$ for plant height and leaves above the ear, it is expected that manipulation of plant height for reduced plant stature, and leaves above the ear for optimum number, might prove useful in increasing the efficiency of plants with regard to their harvest index in these populations.

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STUDIES ON MAIZE GENE POOLS. II. HERITABILITY AND EXPECTED GENETIC ADVANCE[1]

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Heritability estimates were made in two gene pools of maize following Hallauer and Miranda (1981). In both the pools, days to silk recorded higher heritability; whereas moderate values were recorded for plant height, ear height, leaves above the ear and ear length, and variable results were obtained for other characters including yield per plant. Further, the yield components, viz. ear length, ear diameter and kernel rows per ear recorded lower estimates of genetic advance as compared to yield itself, suggesting that in these populations significant improvement in yield can be obtained by directing selection for yield per se and there is possibility of achieving actual gains of the order of around 5%. Selection based on full-sib progenies was superior to mass selection. Exploitation of improved gene pools through heterosis breeding is suggested for obtaining quantum jump in yield levels of early maturing maize hybrids.

Keywords: expected genetic advance, gene pools, heritability, *Zea mays*

Introduction

In a given population, heritability provides a measure of additive genetic variation on which selection depends. This value alone does not have much significance as it fails to account for absolute variability, and therefore, heritability in conjunction with selection differential indicates the expected genetic gain resulting through selection. Keeping in mind the importance of developing early maturing varieties through population improvement, these estimates were made in two early maturing, heterozygous base populations (gene pools) developed recently under the All India Coordinated Maize Improvement Project.

Materials and methods

The materials and the experimental layout/details used in the present study were earlier described by Arha, Sarda and Agarwal (1988).

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Heritability estimates were made from the components of variance for each character in both the environments (E_1 and E_2) separately and over the environments following Hallauer and Miranda 1(981). Expected genetic gains from selection of the highest yielding 5% of the units for full-sib family selection were calculated following the procedure outlined by Robinson et al. (1949). An approximate procedure (Goodman, 1965) was used to estimate the expected gains from mass selection.

Results and discussion

The heritability estimates (narrow sense) for the two populations (P_1 and P_2) are summarized in Table 1. The estimates for different characters varied from 16.04 to 94.13% when both the populations and the three analyses, viz. E_1, E_2 and combined, were taken into consideration. The estimate of

Table 1

Heritability (narrow sense), and expected genetic advance (percentage of mean) from one cycle selection through full-sib family selection (F. S.) and Mass selection (M. S.) for P_1 and P_2

Character	Environment	Heritability		Expected genetic advance			
				P_1		P_2	
		P_1	P_2	F. M.	M. S.	F. S.	M. S.
Plant height (cm)	E^1	72.68	61.47	9.42	3.05	11.70	4.90
	E^2	88.82	87.12	20.73	8.84	18.34	7.61
	Combined	52.81	59.18	10.28	4.04	13.19	5.49
Ear height (cm)	E^1	33.12	66.38	10.57	3.39	18.43	6.31
	E^2	83.73	87.28	32.98	12.71	30.77	12.89
	Combined	41.08	55.96	13.80	5.09	20.48	8.09
Leaves above the ear	E^1	71.49	76.34	7.02	2.25	8.12	2.75
	E^2	73.97	57.21	8.53	2.69	5.20	1.44
	Combined	53.28	45.64	6.05	1.96	4.40	1.32
Ear length (cm)	E^1	48.13	68.88	5.07	1.41	12.36	4.62
	E^2	48.19	60.86	10.05	3.93	12.00	4.29
	Combined	43.76	64.42	4.95	1.66	12.44	4.56
Ear diameter (cm)	E^1	56.13	30.75	5.10	1.50	4.64	1.63
	E^2	53.38	53.70	5.21	1.62	5.54	1.66
	Combined	50.79	39.64	4.51	1.36	4.09	1.32
Kernel rows per ear (No.)	E^1	52.81	16.04	4.62	1.30	2.77	1.02
	E^2	16.35	44.85	1.65	0.47	5.98	2.00
	Combined	41.98	30.18	2.99	0.85	3.22	1.12
Days to 50% Silk (No.)	E^1	91.10	93.36	12.23	5.76	12.39	6.71
	E^2	94.13	88.78	11.20	6.14	8.84	3.78
	Combined	69.92	75.54	10.72	5.42	9.73	4.67
Yield per plant (g)	E^1	36.98	67.55	7.87	1.88	22.25	6.68
	E^2	62.63	31.70	20.76	6.82	11.94	3.56
	Combined	28.92	55.10	5.52	1.58	15.63	4.68

heritability and genetic advance for leaves above the ear and days to silk are high and of the same order in E_1 for both the populations. These estimates for plant height, ear height, ear diameter and days to silk are of the same order in both the populations in E_2. In P_1, the estimates of heritability are inconsistent in both the environments for ear height, kernel rows per ear and yield per plant; and in P_2 these are inconsistent for plant height, ear height, leaves above the ear, ear diameter, kernel rows per ear and yield per plant.

Since the estimates of heritability were inconsistent over the environments for most of the characters it seems useful to consider combined analysis to deduce more realistic conclusions. In both the populations, days to silk recorded high values of heritability; moderate values were recorded for plant height, ear height, leaves above the ear and ear length. However, ear diameter and kernel rows per ear recorded moderate values of heritability in P_1 and low values in P_2 while yield per plant recorded a low level of heritability in P_1 and a moderate level in P_2. Thus, it should be possible to affect genetic improvement through selection for most of the traits under study and that moderate to high intensity of selection would be most effective for making rapid gains.

The estimates of the expected genetic advance from the combined data should provide information of relatively more general applicability (Table 1). In general, for both the populations, the predicted gains for full-sib family selection indicated that plant height, ear height and days to silk recorded relatively higher genetic gains as compared to those of recorded for leaves above the ear, ear length, ear diameter, kernel rows per ear and yield per plant, except that values for ear length and yield per plant in P_2 were comparable with the former group. Thus the estimates, revealed that yield components, viz. ear length, ear diameter and kernel rows per ear, recorded lower estimates of genetic advance as compared to yield itself, suggesting that in these populations significant improvement in yield can be obtained by directing selection for yield *per se*. This observation concurs with that of Hallauer and Miranda (1981) wherein, from a large body of data on correlations between yield and yield components, they concluded that the most effective method for yield improvement in maize seems to be direct improvement of yield itself.

In a recent study, Batta et al. (1981), taking one population of maize, reported greater genetic advance under non-stress environment for grain yield and days to 50% silking whereas for ear length, plant height and ear height stress environment was indicated to be more suitable for selection. In the present investigations wherein two populations have been studied, this kind of specificity does not seem to exist. We shall consider the relevant part of the data from the present study to make the point clear. In the present study, E_1 was less favourable environment as compared to E_2 (more favourable environment) as revealed by the mean and range values recorded for different char-

acters in the two populations. In both the populations, plant height and ear height recorded higher gains in E_2. However, no definite increasing or decreasing trend of genetic gains was observed for kernel rows per ear and yield per plant with respect to two environments in two populations, which clearly indicates a lack of specificity of environment for obtaining greater genetic advance for a specific trait.

The expected genetic gains for yield per plant in P_1 through full-sib family selection were 7.87% in E_1, 20.76% in E_2 and 5.52% in the combined analysis. Similarly in P_2, the expected gains for yield per plant were 22.25% in E_1, 11.94% in E_2 and 15.63% in the combined data. These values indicated the possibility of achieving actual gains of the order of around 5% per cycle in both the populations. Since C. V. per cent and g×e interactions were of lower magnitude, the discrepancy between the realized and the predicted gains is expected to be low. Slower genetic progress is expected in P_1 as compared to P_2 and consequently the former population is likely to require more number of cycles of selection, as compared to the latter, to realize the same amount of gains.

Also, Table 1 indicates that in both the populations, predicted genetic gains for yield per plant from full-sib family selection were about 2 to 3 times more than from the mass selection. These results agree with Prodhan et al. (1981) and Sanghi (1983).

In our earlier study (Arha, Sarda and Agarwal, 1988) manipulation of plant height and leaves above the ear was suggested for increasing efficiency of plants with regard to their harvest index in these populations. Considering values of heritability and genetic advance recorded for these characters it is expected that selection for these traits would be equally effective. However, in order to achieve rapid and greater genetic gains for harvest index, relatively more emphasis should be placed on manipulation of plant height for short stature as compared to that of optimization of number of leaves above the ear, as values of expected genetic gains are higher for the former trait when compared to those for the latter one.

Currently, these populations are undergoing intrapopulation improvement in the maize project. The improved versions can be used as commercial composite varieties. However, subsequent exploitation of these populations in specific hybrid combinations to ensure development of superior hybrids of early maturing range is suggested. In fact, exploitation of such improved broad-based populations (gene pools) through heterosis breeding should become one of the important prepositions in future years for obtaining a quantum jump in yield levels of early maturing maize hybrids.

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INHERITANCE OF THE RATE OF GERMINATION AND EMERGENCE AT LOW TEMPERATURES IN MAIZE (*ZEA MAYS* L.)

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In the case of lines and F_1 hybrids of 10×10 complete diallel crosses the rate of germination, emergence and growth of seedlings at 10 °C under aseptic conditions was studied. For all three properties epistatic effects were indicated in inheritance. The additive genetic variance is prevalent compared to the dominance and the epistasis. Selection can be started in an early phase of breeding. There is a considerable maternal effect in the linear and reciprocal crosses.

Germination has no correlation either with emergence ($r = 0.029$) or with the growth of seedlings ($r = -0.132$). On this basis emergence can be divided in two parts: germination and growth of seedlings. The inheritance of germination is independent from that of the growth of seedlings, which makes it possible to combine favourable characteristics by crossing suitable lines.

Keywords: cold, diallel cross, emergence and growth of seedlings, maize, germination

Introduction

The spring temperature usually is low enough to have an unfavourable influence on the germination and emergence of thermophilous plants such as maize. Despite this, maize sowing must be started in April when the temperature of soil reaches 8–210°C. Maize production therefore requires such hybrids the seed of which quickly germinates at that temperature and grows vigorously.

The minimum germination temperature for maize is 5 °C in the case of cold-tolerant lines and hybrids (Koch, 1971), though it may greatly vary with the genotype (Blacklow, 1972, McConnel and Gardner, 1979, Maryam and Jones, 1983). There are considerable differences in the rate of germination between genotypes evenat identical temperatures (Maryam and Jones, 1983, Eagles and Hardacre, 1979).

The minimum temperature of emergence is about 9 °C for cold-tolerant maize lines and -hybrids and, as in the case of germination, shows dependence on genotype (Koch, 1971). Several authors observed genetic differences in the time of emergence at low temperatures (Herczegh, 1978, Eagles and Hardacre, 1979, Szundi and Kovács, 1981, Eagles 1982).

The emergence consists of two distinct phases: germination and post-germination growth of plant.

We have genetic knowledge of germination and emergence separately (Eagles, 1982). In the case of in vitro germinated genotypes Maryam and Jones (1983) found the additive dominance model to be adequate at 10/6 °C. The high inheritability value taken in a narrow sense shows a fair chance of successful selection for rate of germination. McConnel and Gardner (1979a) made progress of about 8% per cycle in selecting for germination at 7,2 °C, though at that temperature they also detected an epistatic effect in inheritance (McConnel and Gardner, 1979b). Eagles and Hardacre (1979) observed dominant and epistatic gene effects in the inheritance of the time of emergence. Some reported a maternal effect when studying the germination and emergence (Gubbels, 1974, Bose et al. 1982). On the other hand, there is no answer yet to the question of the relation between the rates of germination emergence growth of seedlings.

It is difficult to acquire a knowledge of the rate of embryonic plant growth, since the beginning of this process — which lasts from the end of germination to emergence — cannot be seen in the soil.

By determining the time of germination and the time of emergence at the same temperature, in the case of lines and hybrids the difference between the two, the time of the post-germination growth of the seedling can be calculated, supposing that germination takes place in the soil the same way as in the Petri dish.

Apart from the genotype the physiological properties of the maize seeds are influenced by the shape of the grains and their position in the ear where they ripened. Flat grains from the middle of the ear show the most favourable properties, so for experimental purposes these are generally used.

The rates of germination and seedling growth are influenced not only by the temperature but also by soil-borne pathogenic microorganisms (Miedema, 1982, Bochicchio, 1985). Therefore when studying inheritance the pathogens must be eliminated and the work possibly done under sterile conditions.

Materials and methods

The cold tolerance experiments were carried out in the phytotron of the Agricultural Research Institute of the Hungarian Academy of Sciences. In the experiment cold tolerance in ten inbred lines (B-14, H-99, N-6, W-153R, W-22R, 156, F-564, WF-9, A-632, HMv-1409), in the F_1 hybrids of a complete diallel cross produced from them was studied. The lines were sown in the field in medium heavy forest soil prepared as if for a garden, at a spacing of 75×25 cm, at three sowing dates in order to achieve simultaneous flowering of the lines of different vegetation periods. For the examination of germination of and emergence 40 grains from the middle of 3 ears of each line and combination were used.

Before germination the grains were sterilized with a 1.5 g/lit. concentration solution of benzol-sulphon-chloro-amide-sodium (neomagnol) for 24 hours. One examining the emergence, the grains were similarly sterilized, then treated with Quinolat V-4-X and sown into a sterilized 3 : 3 : 1 ratio mixture of arable soil, sand and vegasca. Germination and emergence took place under aseptic conditions in G-30 type (Conviron) thermostat, at constant 10 °C in dark.

During the experiment germination was followed every day, emergence on 4 occasion a week.

The basic data were evaluated by analysis of variance (Sváb, 1979). The general and special combining abilities were calculated after Keuls and Garretsen (1977). To establish the genetic variation the model of Hayman (1954a, b), for the analysis of covariance/variance the method of Jinks (1954) was used. Connections between characteristics were described by correlation (Sváb, 1979) in the case of both basic data and combining ability.

Results and discussion

In our experiment the inheritance of the rates of germination, growth of embryonic plant and emergence in lines and F_1 hybrids of 10×10 complete diallel crosses, as well as the correlations of the three characteristics were studied at low temperatures under pathogen-free conditions.

The means of germination time for the parents and their hybrids are seen in Table 1. The genotypes show a considerable variation of germination rate. The two most rapidly germinating lines are: HMv–1409 and A–632, while those slowest in germination: WF–9 and 156. In several combinations the hybrid even germinates sooner than the early parent (HMv–1409 × B–14, F–564 × A–632, WF9 × N–6).

According to the means of emergence time for the parents and their hybrids (Table 2.) the genotypes show a wide variation: 156 and A–632 are the fastest F–564 and HMv–1409 the slowest to emerge. In the F_1 hybrids a considerable negative heterosis can be observed both with combinations consisting of quickly emerging (156 × A–632) and with those consisting of slowly emerging (HMv–1409 × WF–9) lines.

It was on the basis of the difference between the germination time and the time until emergence that we determined the value of the growth of embryonic plant (Table 3). The line show a wider variation than in the case of emergence. The lines 156 and A–632 are the fastest, while H–99 and HMv–1409 are the slowest as regards the growth of embryonic plant. In the F_1 hybrids there is a favourable heterosis effect; it is most intensive in the 156 × A–632 combination, but can be observed in the quick × slow (156 × F–564) and the slow × slow (HMv–1409 × F–564) crosses too. Among the hybrids the 156 × × A–632 and 156 × W–22R combinations are the quickest, and the B–14 × × H–99 and W–22R × H–99 ones the slowest in the growth of embryonic plant.

On the basis of the analysis of variation the general and special combining ability is significant at $p = 0.1\%$ for all three characteristics examined, which suggests that both the additive and non-additive gene effects play an important role in the inheritance of characteristics. Similarly reliable is the general reciprocal effects, so a considerable maternal influence must also be reckoned with. The G.c.a/s.c.a. ratio is high in all the three cases (10.8 for germination 3.1 for emergence and 4.5 for the growth of seedlings which means

Table 1

Mean value of the germination time of parents and F_1 hybrids
(day)

	HMv-1409	A-632	F-564	B-14	W-153R	H-99	W-22R	N-6	WF-9	156	Wr-Vr	Wr+Vr
HMv-1409	8.8	8.9	8.9	7.9	9.6	8.0	8.7	9.2	8.6	9.1	0.26	1.32
A-632	10.4	10.5	9.8	11.6	9.6	9.6	9.9	10.2	8.8	8.8	0.20	1.41
F-564	8.8	9.2	10.5	8.8	8.8	10.2	8.8	10.2	9.7	9.3	0.09	1.41
B-14	9.7	11.1	11.0	11.2	8.7	11.1	11.2	10.7	9.6	9.9	−0.53	2.32
W-153R	10.6	9.4	11.5	10.0	11.4	12.5	12.3	12.9	12.4	8.8	−0.14	2.86
H-99	9.8	11.2	10.4	12.2	10.5	11.8	9.8	11.5	10.9	11.3	0.39	1.98
W-22R	12.0	12.5	11.8	13.0	13.8	12.7	12.8	13.0	15.1	12.4	0.31	2.36
N-6	10.9	10.6	13.1	10.6	11.3	11.4	11.4	13.6	11.8	10.6	0.10	3.25
WF-9	9.3	11.8	8.9	9.4	11.8	12.2	10.5	10.5	14.0	9.9	−0.37	5.14
156	12.7	13.2	12.3	14.2	12.6	11.5	13.23	16.9	15.1	14.3	0.26	3.55
Average											0.06	2.58
Variance											0.09	1.36

$LSD_{5.0\%} = 1.6$
$LSD_{1.0\%} = 2.1$
$LSD_{0.1\%} = 2.7$

Table 2

Mean value of the emergence time of parents and F_1 hybrids
(day)

	A-632	156	W-22R	B-14	W-153R	N-6	H-99	WF-9	F-564	HMv-1409	Wr-Vr	Wr+Vr
A-632	54.8	36.9	38.7	45.8	44.7	41.4	48.9	39.3	46.8	46.6	−17.2	55.1
156	34.1	55.1	34.9	37.6	39.0	42.1	44.4	42.9	39.8	50.5	−103.5	−14.0
W-22R	37.2	62.7	39.7	57.1	46.9	69.4	40.9	45.9	45.7	45.7	−135.6	19.0
B-14	53.5	39.9	39.7	64.9	43.4	48.9	73.5	35.7	54.2	49.5	−38.7	110.0
W-153R	41.5	41.2	46.2	44.6	75.0	48.5	55.2	52.1	42.0	55.9	−263.7	290.9
N-6	40.9	47.8	43.1	42.1	56.2	75.0	56.1	56.5	64.1	53.3	−332.1	263.2
H-99	49.2	49.1	50.7	41.8	54.6	61.0	75.5	64.5	46.5	53.0	−202.4	448.6
WF-9	36.5	43.0	53.1	38.9	53.2	55.7	57.7	75.8	64.2	61.1	−423.1	227.7
F-564	46.7	40.4	41.3	43.7	51.3	56.8	47.7	58.7	80.0	56.1	−241.0	381.9
HMv-1409	40.3	55.6	44.4	43.8	61.4	49.5	58.9	45.5	75.0	81.0	−218.6	399.1
Average											−197.6	218.2
Variance											16 238.9	27 991.1

LSD $_{5.0\%}$ = 6.2
LSD $_{1.0\%}$ = 8.2
LSD $_{0.1\%}$ = 10.6

Table 3

Mean value of the time of embryonic plant growth of parents and F_1 hybrids
(day)

	156	A-632	W-22R	B-14	N-6	WF-9	W-153R	H-99	F-564	HMv-1409	Wr-Vr	Wr+Vr
156	40.8	20.8	21.6	23.3	25.5	28.4	26.3	32.9	27.4	37.8	−23.67	57.91
A-632	27.1	44.3	28.8	34.2	31.2	30.5	35.1	39.3	37.0	37.4	−27.84	47.26
W-22R	24.8	26.8	49.9	26.7	33.9	25.8	43.3	56.7	34.1	33.7	−44.63	106.37
B-14	29.9	42.3	28.5	53.6	38.2	26.1	34.7	62.4	43.2	39.8	−50.87	94.09
N-6	37.1	29.7	31.7	31.5	61.3	44.7	44.8	44.6	50.5	42.3	−24.64	160.81
WF-9	32.2	24.7	43.2	26.9	45.2	61.8	41.1	45.4	55.3	51.9	−41.10	218.33
W-153R	32.4	32.1	33.9	34.1	35.6	39.7	63.6	42.6	30.5	45.3	−32.76	152.39
H-99	37.8	37.9	40.9	29.6	49.5	53.6	44.1	63.7	36.1	43.1	−35.89	95.79
F-564	31.1	37.4	32.5	34.8	46.5	49.2	42.2	37.5	69.9	47.2	−54.98	227.97
HMv-1409	46.5	31.4	35.6	35.8	40.3	36.9	51.8	50.8	48.0	72.7	−34.78	199.64
Average											−37.11	136.06
Variance											114.45	4266.32

$LSD_{5.0\%} = 5.5$
$LSD_{1.0\%} = 7.3$
$LSD_{0.1\%} = 9.4$

that the additive genetic variance is prevalent compared to the non-additive genetic variance, the dominance and the epistasis.

The purpose of breeding is best served by the quickly germinating and emerging plants. Therefore those lines are favourably considered where the general combining ability is negative and the S.c.a. variance is high (Table 4). Accordingly, from the point of view of germination HMv–1409 is the best followed by F–564 and A–632 while 156 and W–22R are worst of the lines examined. In the various combinations the line W–F9 is expected to show the highest variability (on the basis of the s.c.a. variance).

Table 4

	Germination		Emergence		Growth of embryonic plant		Germination GRE	Emergence GRE	Growth of embryonic plant GRE
	GCA	SCAvar	GCA	SCAvar	GCA	SCAvar			
HMv 1409	−1.4	0.47	2.9	4.26	3.8	4.21	−0.7	−1.0	−0.1
F 564	−0.8	0.65	1.3	6.06	1.7	5.59	−0.7	−0.7	−0.1
A 632	−0.6	0.61	−4.6	6.40	−4.8	6.51	−0.5	0.3	0.8
B 14	−0.2	0.91	−1.4	8.01	−1.9	7.82	−0.2	2.9	3.2
W 153R	0.0	0.85	0.7	3.52	−0.2	2.96	0.2	−1.7	−1.9
H 99	0.1	0.55	6.5	4.55	7.0	5.54	0.1	−2.1	−2.0
WF 9	0.2	1.12	1.3	6.06	2.8	7.98	−0.3	1.3	1.6
N 6	0.7	0.71	2.1	4.06	2.7	6.62	−0.1	0.4	0.5
W 22R	0.9	0.55	−1.7	7.09	−3.4	7.19	1.0	1.4	0.4
156	1.0	0.73	−5.4	6.64	−7.2	6.41	1.5	−1.3	−2.8

With emergence the order of the lines totally changes. Now 156 and A–632 are regarded as best, and H–99 as worst. HMv–1409 the best line for germination belongs to the average lines. On the basis of the s.c.a. variance B–14 is expected here to show the highest variability in combinations.

In the case of the calculated parameter: the growth of seedlings the lines 156 and A–632 are the best — just like in the case of emergence, and H–99 and HMv–1409 show the poorest combining ability.

On the basis of the reliable general reciprocal effects (GRE) (Table 4). the lines are evaluated according to the above. Here too, the negative GRE values are considered good. Thus, if early germination is the aim, HMv–1409 and F–564 should be chosen as maternal lines, and 156 which gives the lowest GRE value, as paternal line. As regards emergence H–99 and W–153R are outstandingly better than the others, so on the basis of this parameters they are reasonably chosen as maternal lines, while B–14, due to its high GRE value had better be used as paternal line. According to the GRE values of embryonic plant growth 156, H–99 and W–153R as maternal lines may improve this property, while B–14 should be used, here too, first of all as paternal

line. The examination of the GRE values calls attention to the fact that if a property is to be improved by crossing we must decide in advance on the maternal line. A properly chosen maternal line may offer substantial help in making progress in the selection.

As the next step we performed the analysis of covariance (Wr) — variance (Vr). According to the results there are epistatic effect with all the three properties, so genetic parameters could not be established.

We also examined the correlation of the three properties. Germination is in no correlation either with emergence ($r = -0.029$) or with the growth of embryonic plant ($r = -0.132$). This, and the different orders of g.c.a. suggest that germination is inherited irrespective of the other two properties examined.

On this basis emergence can be divided in two distinct processes: germination and growth of seedlings. The independent inheritance offers a possibility of combining favourable properties in one line. The prevalence of the additive genetic variance with both properties suggests that selection can even be started in an early phase of breeding. When the selection is made in two steps more rapid progress can be achieved than when we only select for quick emergence. Out of the lines examined a good basis would be provided for this by the combinations HMv–1409×156 and HMv—1409×A–632.

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A STUDY OF HETEROSIS IN INDIAN MUSTARD

(*BRASSICA JUNCEA* L. COSS. and CZERN.)

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Thirteen inbred lines/varieties were crossed with three varieties, viz., RH-30, Varuna and Parkash in line × Tester fashion extent of heterosis over midparent value and better parent for a number of morphological attributes in Indian Mustard. The results indicated that the crosses possessing positive heterosis for seed yield had positive heterosis for primary branches, secondary branches, siliqua length and seeds per silique. Highest positive heterosis for seed yield (182.09%) was observed in the cross RLM-198 × × RH-30 followed by the crosses RLM-154 × Varuna (155.5%), RL 18 × Varuna (142.56%) and RS-64 × Varuna (199.98%). The cross RLM-198 × RH-30 also recorded highest heterobeltosis (183.9%) for secondary branches. The characters, siliqua length and oil content recorded marginal heterosis.

Keywords: indian mustard, *Brassica juncea* L. COSS. and Czern., inbreeding, heterosis

Introduction

The per capita availability of fats and oils (>14 g) in India is far below the minimum international requirement of 38 g. This is primarily because of low productivity of oilseeds. In Indian mustard, traditionally pure line cultivars have mostly been bred and limited success has been attained by the breeders in terms of realizing increased yields. One of the potential ways to get a substantial increase in production of Indian mustard is the exploitation of heterosis through development of hybrids. The availability of cytoplasmic male sterility (Rawat and Anand 1979; Brar et al. 1980 and Banga and Labana 1983) and fertility restorer genes have solved the problem of producing hybrid seed commercially. Before initiating a hybrid seed production programme, it is essential to study the significant heterosis that must be encountered in the F_1 hybrid. Considering these facts, the present study was undertaken.

Materials and methods

Thirteen inbred lines/varieties were crossed with three well adapted varieties, viz., RH-30, Varuna and Prakash in Line × Tester fashion to produce 39 crosses, thus constituting a total of 55 treatments. Parents were selected on the basis of diversity for various economic traits and their adaptability under different agroclimatic conditions. The varieties Kranti and Varuna are high yielding types recommended at the national level RK-1467 and RLM-198 for

their high input conditions, RLM-514, for its reinfed conditions, RH-30, for its bold seeds and shattering resistance and B-85 for its white-creamy flowers. Sixteen parents, with their 39 F_1 hybrids, are grown in replicated randomized block design at the Research Farm of the Haryana Agricultural University, Hisar, during 1984–85. Each genotype was represented by a single row of 5 m, spaced at 30 cm between rows and 15 cm between the plants. Five plants in each entry were selected at random in each replication for recording observations on: (1) Primary branches; (2) Secondary branches; (3) Siliqua length (cm); (4) Seeds per siliqua; (5) Seed yield/plant (g) 8nd (6) oil content (%).

Statistical analyses were carried out on the mean values of five plants obtained in each entry. "Heterobeltosis" and "Relative heterosis" were calculated for each character separately.

Results

The heterosis over better parent and mid-parental value for each trait are presented in Table 1. A perusal of data indicated in general that crosses, possessing significant positive heterosis over better parent and mid-parental value for seed yield, had positive heterosis for primary branches, secondary branches, siliqua length and seeds per siliqua.

The highest positive heterosis for seed yield (182.09%) was observed in the cross RLM–198 × RH–30 followed by the crosses RLM–514 × Varuna (155.57%), RL–18 × Varuna (142.56%) RS–64 × Varuna (110.98%), PR–1108 × Varuna (124.74%), and RC–781 × Varuna (102.80%). The hybrid advantage over better parent ranged from 41.89% (B–85) (white flower) × Parkash to 30.16% (RK–1467 × RH–30) for primary branches and from 40.79% (TM–2 × Parkash) to 183.93% (RLM–198 × RH–30) for primary branches and from 40.79% (TM–2 × Parkash) to 183.93% (RLM–198 × RH–30) for secondary branches. A cross RLM–514 × Varuna, exhibiting the maximum increase over secondary branches (89.10%) and seed yield (155.57%). Siliqua length and oil content displayed marginal values of heterosis. The heterosis for siliqua length varied from 31.77% (B–85 (white flower) × Varuna) to 15.66% (TM–2 × RH–30), and for oil content from 10.89% (TM–2 × RH–30) to 8.43% (Australian × RH–30).

The cross B–85 (white flower) × Parkash recorded minimal heterosis for seeds per siliqua (21.61%) followed by the cross TN–7 × Parkash, having 20.47% heterosis. These crosses also had positive heterosis for seed yield.

Discussion

The occurrence of increased seed yield in the F_1 hybrid over the better parent (heterobeltosis) and mid-parental value (relative heterosis) has a direct bearing on increasing the production of mustard. The present study revealed that heterosis for seed yield was maximum among the various traits. Though all the hybrids did not show heterosis, however, a large number of hybrids

outyielded the better parent and mid-parental values (Fig. 1). The hybrids showing heterosis for seed yield were not heterotic for all the characters, but the highest heterosis was manifested in the component traits, which were associated with sed yield. The study of heterosis among individual crosses in this experiment shows that specific hybrids exhibiting high heterosis for seed yield had also shown high heterosis for primary and secondary branches. Similar results have been reported by Singh, 1973, Paul et al. 1976. and Banga and Labana 1984. Several workers, viz., Singh and Singh, 1972; Asthana and Singh, 1973; Yadava et al. 1973; Labana et al. 1975; Rupta, 1976 and Yadava et al. 1985, have highlighted the positive association of primary branches and secondary branches with seed yield.

Heterosis for seed yield ranged from 38.99%, 182.09%, while 77% of the crossess possessed positive heterosis values. Banga and Labana (1984) observed from 76.01% to 262.67% heterosis over the better parent, while Brar et al. (1980) recorded heterosis to the extent of 144% over the commercial check. Low values of heterosis over better parent for plant height were con-

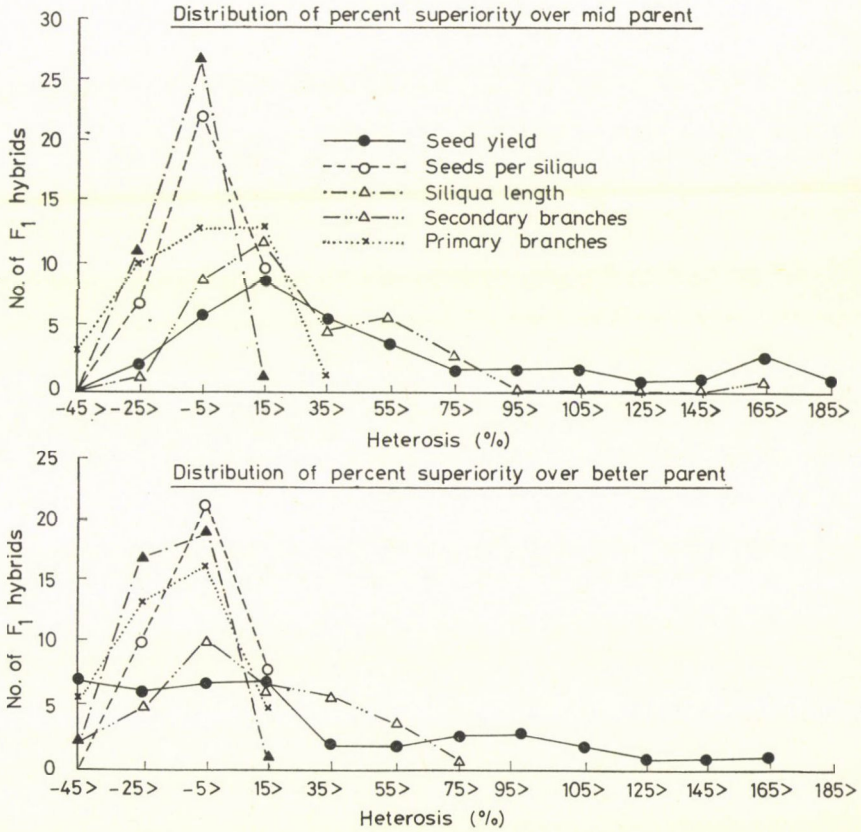

Fig. 1. Frequency distribution of heterosis in Indian mustard

Table 1

Showing heterosis over better parent and mid parent (Parenthesis) for different agronomic traits and oil content

Sr. No.	Cross	Primary branches	Secondary branches	Siliqua length (cm)	Seeds per Siliqua	Seed yield plant	Oil content (%)
1	2	3	4	5	6	7	8
1.	Australian × RH-30	−11.39* (9.38)*	−22.73* (2.41)	−3.47* (2.84)	14.88* (15.98)*	−1.86* (+23.34)*	8.43 (10.53)
2.	RLM-198 × RH-30	12.50* (29.60)*	183.93* (201.42)*	4.80* (5.93)*	−6.07* (−2.33)*	182.09* (192.00)*	1.27 (2.56)
3.	RC-781 × RH-30	11.54* (32.82)*	62.15* (98.6)*	−7.20* (3.26)*	17.22* (16.31)*	31.06* (46.08)*	2.05 (3.33)
4.	PR-15 × RH-30	9.23* (20.34)*	51.79* (61.14)*	−20.52* (−19.47)*	−15.23* (−10.50)*	12.34* (14.49)*	1.68 (2.09)
5.	PR-1108 × RH-30	−23.88* (−15.00)*	−25.55* (19.68)*	−2.07* (0–79)	15.05* (17.59)*	−13.83* (12.50)*	0.84 (1.09)
6.	RL-18 × RH-30	1.43 (−15.43)*	25.48* (46.47)*	4.00 (14.04)*	1.90 (3.06)*	83.87* (86.72)*	−2.48 (0.59)
7.	RLM-514 × RH-30	7.69* (18.64)	69.64* (71.17)*	−3.47* (2.55)*	15.74* (16.25)*	69.35* (51.54)	0.0 (1.45)
8.	B-85 (white flower) × RH-30	−28.30* (−24.00)*	−0.79 (5.44)*	−7.20* (3.26)*	+0.69 (2.28)	23.68* (34.91)*	4.20 (4.87)
9.	RS-751 × RH-30	−2.82 (11.29)*	18.25* (30.12)*	1.07 (7.06)*	−4.87* (1.84)	6.08* (28.96)*	5.44 (5.87)
10.	RH-1467 × RH-30	30.16* (41.38)*	4.20* (16.86)*	2.93* (5.46)*	10.35* (11.36)*	32.85* (96.86)*	4.12 (5.43)
11.	RS-64 × RH-30	−6.45* (0.87)	26.79* (52.0)*	−9.04* (−9.04)*	11.47* (−8.44)*	51.70* (56.75)*	2.90 (3.96)
12.	TM-2 × RH-30	−14.06* (−5.98)	0.25 (38.61)*	15.66* (8.47)*	2.21* (5.57)*	15.45* (47.51)*	−10.89* (−8.88)
13.	TM-7 × TH-30	27.27* (29.63)*	17.86* (22.22)*	6.63* (−8.30)*	−16.57* (21.54)	55.60* (57.88)*	−3.80 (−2.79)
14.	Australian × Varuna	−27.85* (−15.56)*	−29.73* (−5.17)*	−28.00* (−15.97)*	15.34* (23.75)*	−14.26* (15.64)*	1.96 (3.44)
15.	RC-781 × Varuna	−1.28 (14.93)*	19.26* (65.38)*	−12.24* (−5.07)	−11.34* (3.06)*	102.80* (116.21)*	−2.47 (−1.05)
16.	RLM-198 × Varuna	8.33* (21.88)*	−2.82 (9.09)*	−7.29* (−1.46)	4.68* (10.13)*	−18.73* (−1.64)	2.97 (4.06)
17.	PR-15 × Varuna	12.31* (20.66)*	64.22* (72.12)*	4.68* (4.68)*	−10.64* (−8.24)*	19.85* (34.22)*	−2.51 (−1.89)
18.	PR-1108 × Varuna	7.46* (17.07)*	36.05* (52.03)*	−8.03* (−7.79)*	−1.99 (3.93)*	124.74* (157.07)*	5.47 (5.68)
19.	RL-18 × Varuna	17.14* (30.16)*	38.22* (63.16)*	−11.98* (−2.31)	−9.65* (−3.32)*	142.56* (163.31)*	−4.01 (−1.23)
20.	RLM-514 × Varuna	10.77* (19.01)*	89.10* (89.95)*	−7.55* (−0.84)*	−4.36* (3.10)*	155.57* (178.10)*	−0.42 (1.25)

Table 1 (continued)

Sr. No.	Cross	Primary branches	Secondary branches	Siliqua length (cm)	Seeds per Siliqua	Seed yield plant	Oil content (%)
1	2	3	4	5	6	7	8
21.	B-85 (white flower) × Varuna	17.86* (28.16)*	3.15 (11.02)*	−31.77* (−23.17)*	−5.36* (0.95)	89.10* (91.05)	3.81 (4.24)
22.	RS-751 × Varuna	−1.41 (10.24)*	42.34* (58.54)	−11.46* (−5.03)	2.76* (14.47)*	−38.99* (−20.09)*	−0.40 (0.23)
23.	BK-1467 × Varuna	−1.59 (4.20)*	51.75* (72.22)*	−6.77* (−3.24)*	−6.23* (2.77)	101.80* (162.56)*	0.00 (1.47)
24.	RS-64 × Varuna	9.68* (15.25)*	32.11* (46.19)*	−3.91* (−2.89)*	11.79* (24.80)*	110.98* (125.29)*	3.30 (4.59)
25.	TM-2 × Varuna	−28.13* (−23.33)*	3.67* (13.57)*	−15.10* (−8.94)*	−21.83* (−9.25)*	43.97* (70.15)*	−5.64 (−3.29)
26.	TM-7 × Varuna	8.93* (9.91)*	22.94* (25.82)*	1.56* (4.56)*	7.21* (9.67)*	79.20* (94.68)*	−2.14 (3.00)
27.	Australian × Parkash	−13.92* (−12.59)*	−30.18* (16.67)	−3.95* (−2.77)*	4.94* (12.69)*	32.18* (47.01)*	−8.05 (−4.00)
28.	RLM-198 × Parkash	27.78* (26.03)*	44.70* (70.30)*	−16.12* (−10.50)*	−7.03* (4.40)*	3.40 (5.32)*	−5.21 (9.15)
29.	RC-781 × Parkash	−12.16* (−14.47)*	−21.47* (−15.50)*	−5.00* (−1.94)*	15.33* (25.84)	4.34* (8.29)	2.41 (3.06)
30.	PR- × Parkash	−5.41* (0.72)	64.47* (99.20)*	−11.43* (−3.40)*	11.61* (14.71)*	97.74* (123.45)*	2.81 (4.92)
31.	PR-1108 × Parkash	−1.35* (3.55)	−35.52* (10.38)*	−9.59* (−1.13)*	17.36* (24.57)*	−18.37* (12.38)*	3.61 (3.70)
32.	RL-18 × Parkash	−13.51* (−11.11)*	−15.29* (13.92)*	−0.63* (1.27)*	2.57* (9.87)*	19.62* (39.24)*	1.20 (1.39)
33.	RLM-514 × Parkash	−18.92* (−13.67)*	−26.97* (15.27)*	0.30* (1.84)*	14.31* (−7.91)*	3.46 (20.16)*	0.39 (1.39)
34.	B-85 (white flower × Parkash	−41.98* (−29.93)*	−20.39* (15.29)*	12.50* (16.51)*	21.61* (29.84)*	12.89* (39.58)*	0.00 (3.11)
35.	RS-751 × Parkash	−16.22* (−14.48)*	−23.68* (19.72)*	5.21* (7.06)*	5.03* (5.88)*	−3.23* (3.45)	2.41 (4.50)
36.	RK-1467 × Parkash	−12.16 (−5.11)	−30.26* (28.14)	0.56* (5.92)*	0.51 (10.25)*	−9.25* (44.16)*	4.82 (6.09)
37.	RS-64 × Parkash	−12.16* (−4.41)*	−11.84* (11.67)*	−11.70* (−4.60)*	−4.85* (6.32)*	2.64 (21.25)*	−2.41 (−11.03)*
38.	TM-2 × Parkash	−35.14* (−30.43)*	−40.79* (25.62)*	2.11* (3.99)*	−20.65* (−7.80)*	−38.30* (13.03)*	−4.41 (−4.23)
39.	TM-7 × Parkash	−12.16* (0.78)	1.32 (20.31)*	−18.78* (−23.78)*	20.47* (−23.25)*	12.08* (30.36)*	−6.43 (−3.14)

* Significant at a 5% level of significance

trary to those of Banga and Labana (1984). The crosses showing positive heterobeltosis for main shoot height had a positive heterosis for siliquae on the main shoot, and consequently positive heterosis for seed yield in these crosses.

The cross B-86 (white flower) × Prakash recorded the maximum heterosis over better parent (21.61%), but Sagwal (1982)recorded the maximum of 46.07% heterosis for seeds per siliqua. Gupta (1976) had also observed low values of heterosis for this character in his material. Lower values of heterosis for oil content have been observed in the present study. The maximum heterosis (B-43%) has been recorded in a cross Australian × RH-30. Banga and Labana (1984) have reported the maximum of 5.84% heterosis for oil content. Lower values of heterosis for oil content might be due to the lack of genetic diversity for oil content in parent varieties. In the present study, the best hybrid RLM-198 × RH-30 possessed maximum heterobeltosis for seed yield (182.09%), and secondary branches (183.93%).

The results of the present study supported by several workers have clearly indicated the presence of an appreciable amount of heterosis for seed yield and some of the component traits. The exploitation of F_1 hybrid vigour as commercial hybrids in India Mustard will not only help to improve the stagnating yield levels but may also impart stability in production, on account of the well-known heterozygous advantage of the F_1 hybrids over a wide range of environments and their tolerance to disease and insect pests. With the discovery of cytoplasmic male sterility ir *B. juncea* (Rawat and Anand, 1979; Brar et al. (1980) and Banga and Labana (1983)) and known sources of its pollen fertility restoration, the development of hybrids in Indian mustard and the other digenomic species (viz., *B. napus* and *B. carinata*) appears to be feasible in the near future.

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Animal physiology and biochemistry

THE MINERAL STATUS OF RUMINANTS, I. Ca-, P-, Mg- K-, N-, AND Fe CONTENTS IN FEEDSTUFFS

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With the aid of test plants (alfalfa, red clover, wheat, rye) the authors determined the Ca-, P-, Mg-, K-, Na- and Fe contents of fodder plants grown on soils of different geological origin, and (established) the degree in which the animals were supplied with these elements. They followed the changes occurring with the development of plants during the vegetation period using various grass species and -variaties and the above test plants, respectively.

The authors found that irrespective of the soil conditions Ca-, Mg-, K and Fe deficiencies — apart from extreme cases — were not practically to be expected in the feeding of cattle. The P contents of young green feeds and of fodder grains would be enough to cover the minimum requirements, but in other kinds of fodder, byproducts and senesced roughages the quantity of P is very low, therefore feed additive is necessary almost without exception, particularly in calf rearing and in the intensive meat- and milk production. The Na contents of fodders (crops) do not cover the requirements even on soil yielding crops rich in Na.

Keywords: Ca-, P-, Mg-, K-, Na-, Fe status (-supply), indicating plants, addition, minimum requirement, soil type

Introduction

The mineral intake of ruminants shows a much greater fluctuation than that of the monogastric animals. Namely, the mineral supply to the monogastric livestock is determined by the large amount of fodder grains consumed by them. The mineral concentration of grains, tubers, thickened root parts is much more stable than that of the vegetative plant parts (leaf, stalk, etc.) which form the basis of the feed supply of ruminants. In the case of cattle, sheep and horse the kind of plant fed, its stage of vegetation and the geological characteristics of the place of its origin have a decisive role as regards the supply of essential elements. It is at the same time a fundamental rule that in the course of senescence the mineral — and trace element content of the plant decreases, and that the mineral content of the different plants is a specific characteristic of the species (Régius-Mőcsényi and Várhegyi 1978, Régius-Mőcsényi and Szentmihályi 1975).

Materials and methods

For testing the mineral status of ruminants we carried out a nation-wide survey in the framework of a COMECON cooperation to determine the mineral contents of fodder plants. In our present work we examined fodder plants. In our present work we examined fodder plants grown on areas with different soil conditions for Ca-, Mg-, P-, K-, Na- and Fe content, using the following indicator plants: alfalfa *(Medicaco sativa)* collected in bud from grassland, red clover *(Trifolium pratense* var. *spontaneium, Trifolium pratense* var. *sativum)* from grassland and field cultivation, respectively, as well as rye *(Secale cereale)* in flower, and wheat *(Triticum aestivum)* at the beginning of shooting. In choosing the indicator plants was taken into consideration their general occurrence in the different COMECON-countries to be comparable the vegetation of regions with different conditions.

To follow the changes taking place in the quantities of the different elements during the development of the plants we took samples every second week from the first growths of the indicator plants. The changes of a whole vegetation period were registered by a three-year examination of grasses grown on the same soil.

The results obtained in Hungary are compared below with the corresponding results of the GDR where examinations of this character have been widely carried out, while in the other COMECON countries they are now in process.

Results and discussion

The mineral contents of the 5 indicator plants collected from production areas with different soil conditions showed fluctuations depending on the place of origin. Table 1 shows the relative values of Ca-, P-, Mg-, K-, Na and Fe contents in the indicator plants. The calculations were made as follows: the highest average mineral contents of the indicator plants was taken for 100% and the Ca-, P-, Mg-, K-, Na- and Fe contents of plants grown on various soil types were compared to that. In the column "rich" those soils were placed on which grew the indicator plants containing most of the element concerned, while in the column "poor" the opposite is found; the column "rate of fluctuation" shows the percentage difference between the two extreme values. In the material analysed the geological origin and the soil conditions did not essentially influence the P-, Ca- and K-contents of the use of a fertilizer containing these elements. The effect of the geological origin was better felt in the Mg-, Na-indicator plants, as was expected because origin was better felt in the Mg-, Na- and Fe contents of the indicator plants.

In Hungary the vegetation richest in Ca is found on loess while the one poorest in Ca on alkali soils. The difference between the two vegetations is 26%. In the GDR the difference is smaller, a 17% only.

The Ca-contents of the indicator plants (Table 2) are identical in the two countries except the alfalfa and the red clover grown in arables are identical in arables.

In the plants the Ca-content decreases parallel with the age as shown in Fig. 1 on the basis of the results of several years of experiments with 10 grass species (varieties). The second growth is the richest in Ca, in the third growth the Ca-content is lower again.

Table 1

Fluctuations in the mineral contents of fodder crops grown on different types of soil

Element		Soils with indicator plants		Difference* %
		rich	poor	
		in elements		
Ca	GDR	Calcareous soils	Diluvial sand soils	17
	Hungary	loess soils	alkali soils	26
P	GDR	Rothliegendes weathering soils	Upper Triassic weathering soils	15
	Hungary	fen soils	andesite soils	28
Mg	GDR	Rothliegendes weathering soils	Calcareous soils	32
	Hungary	fen soils	andesite soils	20
Na	GDR	fen soils	Phyllite weathering	34
	Hungary	alkali soils	fen soils	31
K	GDR	Rothliegendes weathering soils	Alluvial soils	23
	Hungary	alkali soils	Calcareous sandsoils	20
Fe	GDR	Rothliegendes weathering soils	Clayey soils	32
	Hungary	alkali soils	Andesite soils	27

* Differences between the highest and lowest average relative values of the indicator plants.

Table 2

Ca contents of various green indicator plants in Hungary and in the GDR
(g/kg dry matter)

Plant species	n (Hung; GDR)	Hungary		GDR		%*
		$\bar{x}$	s	$\bar{x}$	s	
Red clover (grassland)	(20; 685)	17	5.3	17	5.3	100
Lucerne	(91; 24)	14	3.7	18	5.0	78
Red clover (field)	(50; 1232)	12	3.3	16	3.8	75
Wheat	(184; 336)	1.4	0.49	1.3	0.38	108
Rye	(68; 259)	1.1	0.46	1.1	0.31	100

* GDR = 100%, Hungary = ×

The P-content of the indicator plants was highest at the beginning of the vegetation period and decreased significantly in the course of the development of the plants (Table 3). In the phase suitable for feeding (Table 4) the P-contents of all indicator plants — except the red clover of grassland origin — were significantly lower in the GDR than in Hungary.

The P-contents of the plant species examined varied in a lower measure than the Ca- and Mg-contents. The leguminous plants are generally richer in

Table 3

Phosphorus content in the indicator plants depending on the vegetation stage (g/kg d.m.)

Plant species		April 9	April 21	May 4	May 18	P	Lowest difference
Lucerne	$\bar{x}$	5.2	4.9	3.9	3.6	<0.001	1.0
	s	0.2	0.2	0.1	0.6		
Red clover from field	$\bar{x}$	5.1	4.8	4.4	2.0	<0.001	0.5
	s	0.3	0.3	0.9	0.2		
Red clover from grassland	$\bar{x}$	—	3.7	2.3	1.6	<0.001	1.5
	s	—	0.6	0.3	0.1		
Rye	$\bar{x}$	6.4	5.7	4.0	2.6	<0.001	2.0
	s	0.2	0.1	0.5	0.5		
Wheat	$\bar{x}$	5.6	5.0	4.6	2.9	<0.001	1.0
	s	0.8	0.4	0.3	0.2		
Meadow fescue	$\bar{x}$	5.4	4.8	4.2	2.5	<0.001	2.0
	s	1.0	0.2	0.6	0.2		

Table 4

Phosphorus contents of the indicator plants in Hungary and in the GDR (g/kg dry matter)

Plant species	n (Hung; GDR)	Hungary		GDR		%*
		$\bar{x}$	s	$\bar{x}$	s	
Red clover (grassland)	(21; 1518)	2.1	0.31	2.5	0.67	84
Lucerne	(90; 65)	3.5	0.67	2.7	0.67	130
Red clover (field)	(57; 3269)	3.0	0.71	2.9	0.79	103
Wheat	(198; 546)	3.1	0.88	2.6	0.54	119
Rye	(78; 485)	2.7	0.67	2.3	0.55	117

Table 5

Magnesium contents of the indicator plants in Hungary and in the GDR (g/kg dry matter)

Plant species	n (Hung; GDR)	Hungary		GDR		%*
		$\bar{x}$	s	$\bar{x}$	s	
Red clover (grassland)	(20; 868)	4.7	1.51	4.0	1.2	118
Lucerne	(91; 24)	3.8	1.07	4.6	1.6	83
Red clover (field)	(54; 1645)	4.2	1.01	3.6	1.1	117
Wheat	(184; 322)	1.5	0.45	1.1	0.29	136
Rye	(71; 265)	1.1	0.31	0.88	0.19	125

* GDR = 100; Hungary = x

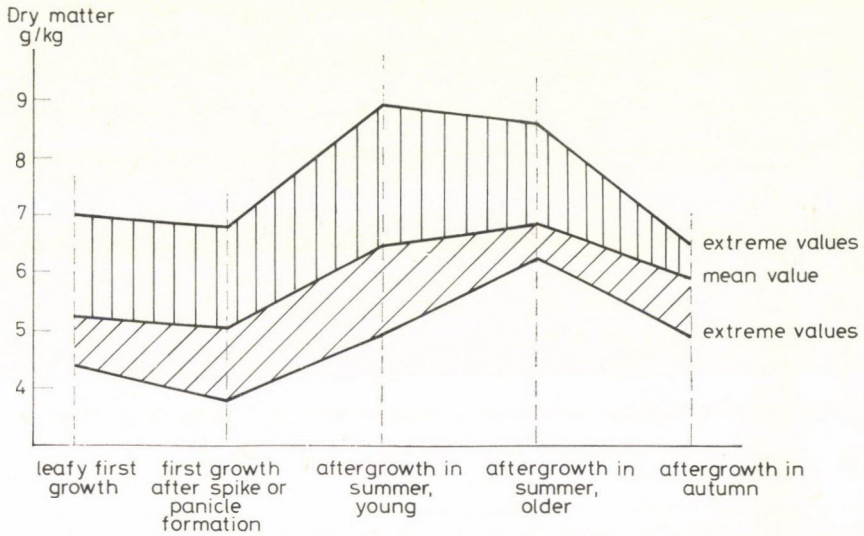

Fig. 1. Trends of changes during vegetation in the Ca-contents of the grass species examined

P than the grasses. The various grasses showed but a slight variation of P-content; the value of the latter ranged between 2.12 and 2.73 g/kg dry matter depending on species and development stage (Régius-Mőcsényi and Várhegyi, 1978, 1980).

The Hungarian indicator plants — except the alfalfa — contained significantly more Mg than those collected in the GDR (Table 5). The Mg-content of a species depends among others on the phase of vegetation, the state of development, the site of growing and the water supply. The cereals are poorer in Mg than the legumes. In the first growth of grasses the Mg-content is essentially lower than in the subsequent ones (Fig. 2). About 2 g/kg Mg is contained in the first growth and it is only at the end of the vegetation period that quantities of 4 g/kg or so are found in the dry matter.

The Na-content of the indicator plants is specific of species and only partly of soil. The vegetation richest in Na is naturally found on alkali soils.

The legumes contain significantly more and the cereals less Na in the GDR than in Hungary (Table 6). Different grasses from the same soil significantly vary in the Na-content of the dry matter throughout the whole vegetation (0.05–1.20 g/kg dry matter, Fig. 3) which again indicates that the Na-content is a characteristic feature of the species.

The geological origin of the soil does not significantly influence the K-content in the plants, though plants growing on alkali soils naturally store more K. The vegetation of Hungary is, in general, poorer in K than that of the GDR, as seen in Table 7, though the difference is significant only in the case of red clover and wheat.

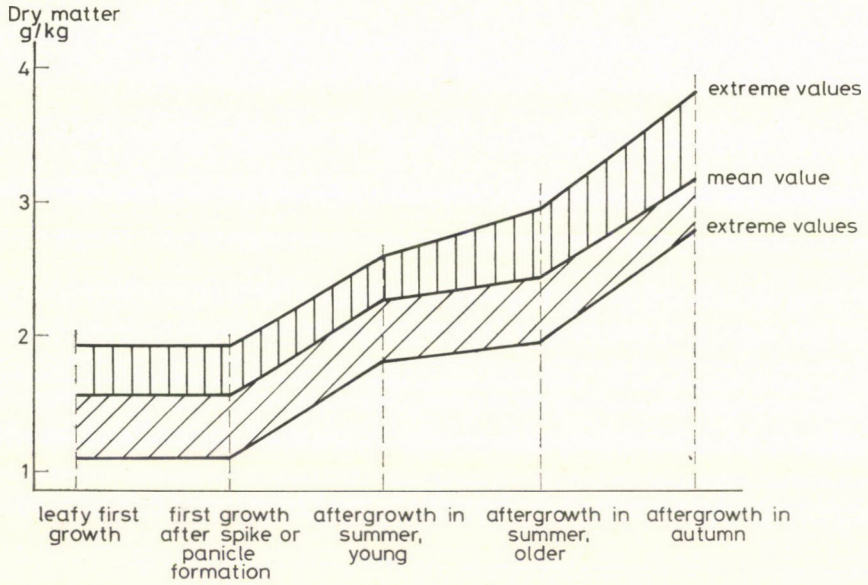

Fig. 2. Trends of changes during vegetation in the Mg-contents of the grasses examined

The K-contents of the grasses are the highest at the beginning of the vegetation period, then decrease with the development of the plants and remain almost permanent in the subsequent growths (Fig. 4).

The tendency of the K-content is opposite to the Mg-content.

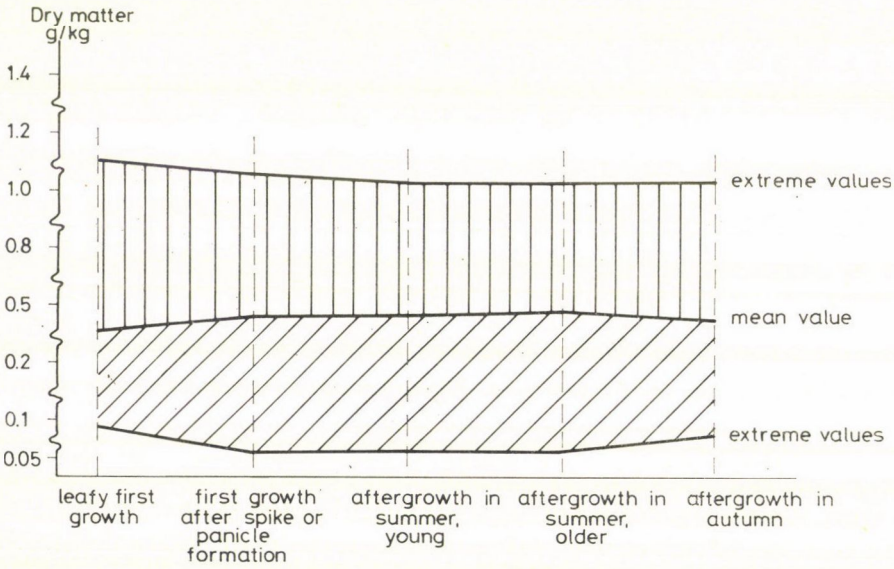

Fig. 3. Trends of changes during vegetation in the Na-contents of the grasses examined

Table 6

Sodium contents of the indicator plants in Hungary and in the GDR
(g/kg dry matter)

Plant species	n (Hung; GDR)	Hungary		GDR		%*
		$\bar{x}$	s	$\bar{x}$	s	
Red clover (grassland)	(20; 1121)	0.29	0.09	0.52	0.40	56
Lucerne	(92; 24)	0.84	0.46	1.1	0.50	76
Red clover (field)	(55; 2924)	0.35	0.24	0.42	0.26	83
Wheat	(184; 338)	0.53	0.30	0.46	0.25	115
Rye	(72; 277)	0.38	0.18	0.32	0.17	119

* GDR = 100; Hungary = x

Table 7

Potassium contents of the indicator plants in Hungary and in the GDR
(g/kg dry matter)

Plant species	n (Hung; GDR)	Hungary		GDR		%*
		$\bar{x}$	s	$\bar{x}$	s	
Red clover (grassland)	(19; 785)	22	6.0	25	13	88
Lucerne	(92; 24)	26	8.8	32	10	81
Red clover (field)	(52; 1894)	30	7.6	35	11	86
Wheat	(180; 329)	26	7.9	28	9.2	93
Rye	(73; 263)	20	5.4	21	6.4	95

* GDR = 100; Hungary = x

The iron intake by the plants precedes the organic matter formation, so it is at the beginning of the vegetation period that the plants contain the largest quantities of Fe which then gradually decrease until utilization (Table 8). Plants of abundant foliage store more iron than those poor in green parts. According to the indicator plants the vegetation of the GDR is richer in iron than that of Hungary (Table 9). In the former country the soil also contains more iron, and the iron intake of plants depends on the iron content of the soil rather than on its pH (Anke et al., 1986; Régius-Mőcsényi et al., 1986).

Irrespectively of the geological origin of the soil and the species and age of the plant the Ca-, Mg-, K- and Fe-requirements for the ruminants and the horse are ensured almost in every case. Exceptions may be the Mg-supply in the case of intensive grassland management, or the Ca-supply when extra large rations of fodder grains are fed. It is often, that Ca-, K-, Mg-, and Fe-content of fodders exceed the requirements of cattle, sheep and horse.

Phosphorus deficiency can be expected first of all with cattle in the stage of intensive development and with cows in milk, especially in the case of deficient feeding in fodder grains. Most green- and mass-fodders do not yet senescent contain about 3.0 g/kg P concerned dry matter.

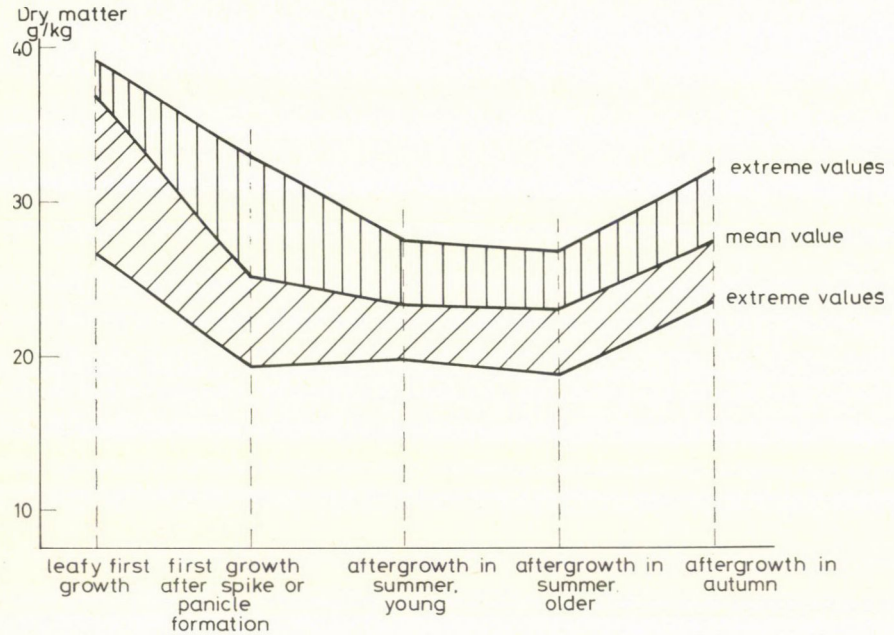

Fig. 4. Trends of changes during vegetation in the K-contents of the grasses examined

In the COMECON-countries 3–3.5 g P per kg dry matter of feed is reckoned with as a minimum maintenance requirement for ruminants.

With the grazing method of keeping the young grass may cover the P-requirements of the animals, but do not in other branches of production (e.g. intensive milk- and meat production).

Table 8

Iron contents of the indicator plants depending on the vegetation
(mg/kg dry matter)

Plant species		April 9	April 21	May 4	May 18	P
Lucerne	$\bar{x}$	171	232	112	115	<0.01
	s	31	70	22	26	
Red clover from field	$\bar{x}$	218	140	104	90	<0.001
	s	15	8	38	29	
Meadow fescue	$\bar{x}$	185	180	81	63	<0.001
	s	37	36	24	5	
Wheat	$\bar{x}$	273	195	102	92	<0.01
	s	120	53	8	8	
Rye	$\bar{x}$	254	148	53	30	<0.001
	s	58	19	5	13	

The utilization of phosphorus in any animal species depends on various factors, such as the quantity of P taken in with the feed, its quality (phytin phosphate, mono- or diphosphate, etc.), the ratio of P to the other elements, the age of the animals, the stage of pregnancy and lactation, etc.

In the dry period of milking cows the P-retention is low, while at the peak of milk production it reaches maximum (Anke and Grün, 1982).

The agricultural useful animals have Ca- and P-reserves in their bones, so an undersupply of these elements does not cause immediately deficiency symptoms. The ruminant fodder rations used in practice result deficiencies mainly of phosphorus.

With an advance in the vegetation period the P-content decreases in the plants; so the senescenced fodders as well as the hoed plants and by-products (beet slice, maize stalk) are poor P-sources. Therefore in cattle- and sheep rearing the P-supplement to the feed ration is necessary in almost every case.

The Na-requirements of cattle, sheep, goat and horse are only covered with large quantities of bet-head or beet slices; any other kind of ruminant- and horse fodder are poor in sodium irrespective of the living space. In spite of the specific effects of the soil there is no growing site where the sodium requirement is fully covered by the amount of sodium contained in the fodder crops. The fodder crops richest in sodium are grown on the alkali soils of Hungary and the moorlands of the GDR. The Na-contents of these crops exceed by 31–34% those of plants grown on other types of soil (Table 1.), still they do not reach a sufficient level to ensure the requirements of the animals even if certain species are able to multiply their Na-contents on the Na-rich alkali soils.

The Na-requirement for the agricultural useful animals ranges from 1 to 2 g/kg dry matter of feed. Cows in milk, in particular those of high milk production are highly responsive to the Na-supply, since a large quantity of Na is excreted with the milk.

With an insufficient supply of sodium the mobilizable proportion of Na in the bones may for a while ensure the quantity of sodium required for the production of milk, but when this reserves is depleted the milk- and butterfat production will be sharply reduced.

Between the Na- and K-metabolism there is an interaction, therefore practical if Na- and K-supply are examined jointly. Their absorption is quick and large in proportion, it may even reach 90%.

To sum it all up, and taking into account the data of the indicator plants examined and the values of requirements it can be established that apart from extreme cases the Ca-, Mg-, K- and Fe requirements for ruminants can be ensured with fodder crops, while P- and Na-supplement are needed almost without exception.

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THE MINERAL STATUS OF RUMINANTS. II. Cu-, Zn- AND Mn CONTENTS OF FEEDSTUFFS AND ANIMAL ORGANS

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The Cu-, Zn- and Mn-contents of fodder crops grown on various types of soil were determined by the authors with the help of indicator plants (red clover, wheat, rye) and of organs (hair, liver, rib bone, cerebrum) the supply of ruminant farm animals.

The authors found that the Cu-deficiency in the ruminants was about 24% on a national level. In the grazing system of keeping this percentage may even be higher owing to the occasional low Cu-content of the grass.

Zn-deficiency occurred in some 11%. The increased Zn-demand of high yielding cows deserves special attention.

The vegetation of loess soils playing a leading role in field crop production, is particularly poor in Mn and requires therefore Mn additions.

Keywords: Cu-, Zn-, Mn-status, indicator organs, indicator plant, minimum requirement

Introduction

The microelement content of fodders depends on the type of soil (geological origin), the plant species, the part of plant (vegetative, generative) and on the age of the plant (Anke 1961, 1968, 1975, 1983; Anke et al. 1971, 1972, 1975, 1980, 1982, 1984; Régius-Mőcsényi and Szentmihályi 1975, 1981; Régius-Mőcsényi and Várhegyi 1983 etc.). The microelement content of the soil — apart from the elements introduced in the soil by fertilization and with occasional industrial contaminations — reflects the microelement content of the base rock. The pH value characteristic of the soil type, which influences the microelement uptake of plants also depends on the base rock (Kovalskij, 1977).

Materials and methods

The Cu-, Zn- and Mn-status of ruminants was examined according to the method of Régius et al. (1987); the elements were determined by atomic absorption spectrophotometer.

Results and discussion

Copper content of the fodders and factors influencing it

The Cu-content in the vegetation of soils of various geological origin was determined with the help of indicator plants (red clover from arable land, *Trifolium pratense* var. *sativum*, and from grassland, *Trifolium pratense* var. *spontaneum; Medicago sativa*; rye, *Secale cereale;* wheat, *Triticum aestivum*). Then the values obtained were relativized and the sequence of the soils was set up according to the relative values for the sake of clarity in the following way: the highest average Cu-content of the indicator plants was taken for 100, and the Cu-contents of the indicator plants grown on the different soil types were compared to this (Table 1). For each soil type the values of 4 indicator plants were averaged.

Table 1

Soil specific Cu-contents of the indicator plants in mg/kg *dry matter, and in a percentage of the Cu-content of plants grown on the soil richest in copper*

Geological origin of soil	Wheat	Rye	Lucerne	Red clover	%
Loess soils	4.2	4.5	8.8	11.0	100
Triassic soils	4.6	4.3	8.2	11.0	97
Andesite soils	4.0	3.2	8.9	12.0	97
Alluvial soils	4.3	4.7	8.5	9.9	93
Alkali soils	4.1	3.8	6.4	11.0	86
Acidic sandy soils	3.7	4.0	7.7	9.0	83
Fen soils	2.5	3.2	7.5	8.3	73
Calcareous sandy soils	3.2	4.4	4.9	7.6	68

In Hungary the vegetation of the loess- and alluvial soils — the two soil types dominant in field crop production — ensures a good copper supply for ruminants. On the acidic sandy and fen soils which are less important from the point of view of agricultural production are grown crops poorer in copper.

In the course of development the Cu-content decreases in the plants, as seen in Table 2. During the 6-week examination period the Cu-content decreases by 37.7% in red clover, by 42.8% in meadow fescue and by 65.5% in rye.

To follow the changes in the Cu-contents of the different grasses during the vegetation period we examined 10 grasses over three years, the first growth in an early, leafy stage and at the time of earing, the second growth similarly on two occasions: when young and in an advanced stage of development, and the third growth once.

Table 2

Changes in the Cu-contents of various fodder crops in the course of vegetation
(mg/kg dry matter)

		9 April	21 April	4 May	18 May	P
Red clover	$\bar{x}$	13.0	12.0	10.0	8.1	<0.01
	s	0.5	0.6	2.3	1.4	
Meadow fescue	$\bar{x}$	11.0	9.1	8.3	6.2	<0.001
	s	1.2	0.6	1.2	1.5	
Rye	$\bar{x}$	8.7	6.0	5.2	3.0	<0.001
	s	0.4	0.9	1.2	1.2	

Table 3 contains the average results of the examinations.

The Cu-content of the rye-grass *(Lolium perenne)* decreased by 31% until the time of earing in the first growth, showed a similar tendency in the second growth, while in the autumn after-growth it exceeded by about 12% the Cu-contents of the first and second growth when young. In the meadow fescue *(Festuca pratensis)*, dactylis *(Dactylis glomerata)*, Hungarian brome grass *(Bromus inermis)*, tall fescue *(Festuca arundinacea)* and reed-grass *(Baldingera arundinacea)* the Cu-content showed similar changes in the course of vegetation; the Cu-content of the timothy *(Phleum pratense)* decreased by half in the second growth; the reduction of Cu in the first growth was some 42% in the blue grass *(Poa pratensis)* and more than 50% in the red fescue *(Festuca rubra)*, and it was 70% in the second growth; in the onion couch *(Arrhenaterum elatius)* the decrease in Cu-content was about 40% both

Table 3

Changes in the Cu-contents of some grasses depending on the stage of development
(mg/kg dry matter)

	Growth I.		Growth II.		Growth III.
	young	after earing	young	older	autumn after-growth
Rye grass	11.3	7.8	11.1	7.0	12.7
Meadow fescue	11.7	8.0	10.2	6.9	12.1
Dactylis	11.8	7.4	11.5	9.8	12.9
Hung. brome grass	12.2	8.3	10.6	8.0	12.9
Tall fescue	10.0	7.7	10.7	8.9	11.6
Reed-grass	13.3	10.0	11.3	9.0	11.9
Timothy	12.9	9.0	11.5	6.0	13.5
Blue grass	12.7	7.4	8.8	6.5	10.6
Red fescue	12.8	6.3	10.9	3.3	9.6
Onion couch	9.3	5.6	8.0	5.0	11.3

in the first and in the second growth. According to the data may ensue so high decrease of Cu-content of different grasses during the senescence, that taking into account the requirement of grazing animals we must reckone with deficiency.

The copper status and the production of animals

According to data of literature (Kronemann et al., 1984, Anke and Risch, 1979) the liver, the cerebrum and the hair are the most suitable of all organs to show the Cu-status. The liver contains 35 mg/kg the cerebrum 9 mg/kg or more Cu per kg dry matter in the case of an adequate supply of copper. The copper content of the hair depends on species and pigmentation; the black cover hair contains 6 mg, the yellow, brown and red ones containe 5 mg or more copper per kg dry matter in the case of a sufficient supply of Cu.

Parallel with collecting the indicator plants we took hair samples from cows kept on areas with similar soil conditions, and on culling organ samples (liver, cerebrum, rib bone) in slaughterhouses. In Table 4 are included the average copper contents of hair, liver and cerebrum from cows kept on four types of soil. The percentage proportion of deficiency is given on the basis of Cu content 5.0 mg/kg dry matter. According to the results obtained the Cu-supply of the andesite soils is insufficient as supported by the quantities of copper detected in the liver and cerebrum. The hair contained 4.9 mg/kg dry matter Cu on an average, and 6% of the Cu-contents of the samples analysed were below 5 mg/kg dry matter; in the liver the average quantity of copper was 19 mg — instead of the extreme 35 mg value — per kg dry matter, and the cerebrum contained 7.9 mg Cu instead of 9 mg/kg in the dry matter. Out of the hair of cows kept on sandy soils 56% contained less than 5 mg copper per 1 kg of dry matter, while the Cu-content of the liver exceeded the average value of 35 mg/kg dry matter which shows the deficit but with the average

Table 4

Cu-contents of hair, liver and cerebrum from cows kept on areas with different soil conditions (mg/kg dry matter)

Soil type	Hair		Liver		Cerebrum		Deficiency %*
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	
Loess soils	7.5	2.5	148	97	17.0	6.7	18
Alkali soils	6.9	2.0	150	81	14.0	4.9	21
Sandy soils	5.6	3.3	79	116	8.4	4.7	56
Andesite soils	4.9	1.5	19	26	7.9	1.4	86

* The deficiency value means the percentage of cows containing less than 5.0 mg Cu/kg dry matter in the hair

8.4 mg/kg dry matter Cu-content of the cerebrum with the wide scatter confirmed the deficiency indicated in the hair. The Cu-supplies of the loess- and alkali-soils are good, the proportion of Cu-deficient hair samples was about 20%.

The coppper requirement may be influenced by a number of antagonistic elements, and under their influence the quantity of copper in the different indicator organs may also change.

The animal species give different responses to larger quantities of copper.

Under experimental conditions 50—200 mg Cu per kg dry matter of feed was given to fattening bulls throughout the period of fattening without any harmful effect, moreover, 100 mg Cu/kg dry matter of feed improved — though not significantly — the feed conversion by 5.1 percent and the live weight gain by 7.4%. The copper content of the hair rose from 9.3 to 15 mg, and with 200 mg Cu fed to 18 mg; the quantity of Cu stored in the liver increased from 45 mg to 176 and 204 mg, respectively.

The zinc contents of fodder crops and the factors influencing them

The zinc contents of plants — like the other macroelements — depend on the geological origin of the soil, the species and age of the plant, the part of plant, the vegetation period, the industrial contamination, the rate of fertilization, the pH of the soil, etc.

Table 5

Soil specific Zn-contents of the indicator plants in mg/kg dry matter and in a percentage of Zn-contents in plants grown on the soil richest in zinc

Geological origin of soil	Wheat	Rye	Lucerne	Red clover	%
Triassic soils	25	20	33	32	100
Alkali soils	18	20	25	39	93
Calcareous sandy soils	20	22	29	28	90
Acidic sandy soils	23	18	27	23	88
Andesite soils	17	14	30	32	85
Fen soils	14	15	31	33	85
Alluvial soils	19	18	24	30	83
Loess soils	18	18	27	24	79

Table 5 shows the soil-specific Zn-contents of the test plants — like in the case of Cu — as a percentage of the highest Zn — content found in the soils examined. According to the values obtained the vegetation of the Triassic and calcareous sandy soils is rich in Zn, while that of the alluvial- and loess soils is poorer.

Table 6

Changes in the zinc-contents of various indicator plants in the course of vegetation (mg/kg dry matter)

Plant species		9 April	21 April	4 May	18 May	P
Lucerne	$\bar{x}$	39	36	38	30	<0.05
	s	2	4	5	2	
Red clover from arable land	$\bar{x}$	46	45	39	30	<0.01
	s	7	3	7	6	
Red clover from grassland	$\bar{x}$	—	44	36	25	<0.001
	s	—	2	5	3	
Rye	$\bar{x}$	43	35	28	20	<0.001
	s	2	6	4	4.1	
Wheat	$\bar{x}$	31	31	30	21	<0.01
	s	4	5	4	2	
Blue grass	$\bar{x}$	45	38	34	22	<0.001
	s	5	2	10	4	

In Table 6 the age-dependent Zn-contents of the test plants can be seen. During the six-week examination the Zn-content decreased by 23% to 32% in wheat, 35% in cultivated red clover, 43% in grassland red clover, 51% in blue grass and 53% in rye. Hence the mineral — and Zn-content, respectively, decrease with the age of the plant.

The average Zn-contents of grasses grown on identical soils (Table 7) show a sharp reduction in the first growth, while in the second growth does

Table 7

Changes in the Zn-contents of grasses from the same place in the course of development and during the vegetation period (mg/kg dry matter)

	Growth I.		Growth II.		Growth III.
	young	after earing	young	older	autumn after-growth
Rye grass	37.5	26.3	—	18.8	36.1
Meadow fescue	29.7	24.8	—	18.5	28.9
Dactylis	24.1	21.8	22.9	14.9	28.0
Hung. brome grass	22.3	21.0	—	19.9	34.2
Tall fescue	23.0	20.0	19.4	13.5	22.1
Reed-grass	33.1	23.9	24.8	—	29.7
Timothy	42.4	29.7	26.3	17.6	36.1
Blue grass	35.2	24.0	—	19.2	28.2
Red fescue	30.6	19.4	15.9	13.3	24.4
Onion couch	20.7	15.1	—	15.7	23.8

not essentially change compared to the reduced level; in the autumn growth the Zn-content rises again approaching the level of the first young growth.

The Zn-status of the ruminants first of all depends on the Zn-content of mass fodders, namely the grain fodders are poor in Zn. Consequently, the concentrates for pig and poultry need Zn-supplementing. The necessity of Zn-supplements is increased by the high phytine content in the seeds of legumes, the thyroid inhibiting compounds (glycozinolates) in the extracted meals — first of all in rape meal —, and the calcium supplements usually overdosed in the practice.

With the above taken into consideration it is not enough to examine and know the zinc-contents of the rations, the zinc-status of the animals must be also examined.

The zinc-status and the production of animals

According to the data by Anke and Risch (1979) the zinc-status is best reflected by the rib bone and the hair.

In cattle the difference between the normal- and the deficit level of Zn-content in the rib bone is more than 40% on an average (normal value: 70 mg, limit value 40 mg Zn/kg dry matter), in sheep it is somewhat less, 36%. In the case of hair, wool and bristle, respectively, the difference between the normal and the deficit level of zinc is only 20–25%.

On the basis of external symptoms (parakeratosis) the Zn-deficiency is difficult to identify, particularly with ruminants where skin lesions can hardly be detected under the hair, besides they may be caused by other mistakes of feeding too, e.g. insufficient or bad quality of feed. Besides, the homeostatic control of the organism tries to maintain the normal level of Zn up to the stage of death, which renders the identification of the deficiency much more difficult.

The Zn-status of the animals was determined by the analysis of the Zn-content of hair and rib bone (Table 8). It was found that in spite of the

Table 8

Zn-contents in the hair and rib bone of cows kept on areas with different soil conditions
(mg/kg dry matter)

Soil type	Hair		Rib bone		Deficiency %*
	$\bar{x}$	s	$\bar{x}$	s	
Loess soils	120	25	71	12	12
Alkali soils	111	20	68	17	21
Sandy soils	107	19	67	11	25
Andesite soils	116	20	72	19	5

* The deficiency value means the percentage proportion of cows containing less than 100 mg Zn/kg dry matter in the hair.

lower Zn-content of the indicator plants compared to those in the GDR or other Central European countries, the animals were properly supplied with zinc. Some 10% of the hair samples analysed contained less than 100 mg Zn/kg dry matter, and the Zn-content of the rib bone did not suggest deficiency on any type of soil.

On the basis of the Zn-content of the hair Zn-deficiency can be expected first of all on the loess- and sandy soils, which may be increased by occasional antagonistic effects (e.g. rape meal, Cd-contamination).

Milking and suckling animals are particularly sensitive to Zn-deficiency, since much zinc is excreted with the milk (3.3 mg/kg milk); for animals of high milk production zinc-additions are particularly important.

The zinc-deficiency — both the primary and the secondary one — may cause a lack of appetite, retarded development, keratin-metabolism disorders, dwarfism, underdeveloped testicles and reduced fertility in male animals.

The species give different responses to the Zn-stress; anaemia, lack of appetite, retarded growth, increased mortality, Cu- and Fe-metabolism disorders may arise. The tolerance of animals is relatively high, under experimental conditions appeared symptoms of toxication only at 5000 mg Zn/kg dry matter in rats, piglets and chickens.

The manganese-contents of fodder crops and the factors influencing them

The manganese-status in ruminants depends on the geological origin of the soil, the species and age of the plant, the plant parts, etc. The grain crops and mostly the maize are poor in manganese, while the grasses generally contain much more of it. The Mn-contents of lucerne and silage maize are between those of the grain crops and grasses. The manganese-status in ruminants consuming forage and in horse depends first of all on he manganese-content of the vegetation of their living space.

The Mn-contents of the different plant species are generally higher on soils of acidic pH, since the plants only can take up the bivalent manganese and cannot take up the quadrivalent one. Accordingly, on the loess- and alluvial soils of higher than 6.5 pH the vegetation generally is poor in manganese compared to plants grown on soils of lower pH.

The soil-dependent manganese-supplies of animals were sized up by means of test plants described in the above. The test plants were collected in the same stage of development from everywhere. Their soil-dependent relative manganese-contents are shown in Table 9. Vegetation richest in Mn is found on the andesite- and the acidic sandy soils, while the calcareous sandy-, the fen- and the peat soils yield crops very poor in manganese. The loess-, the alluvial and the alkali soils occupy an intermediate place.

Table 9

Soil specific Mn-contents of the indicator plants in mg/kg dry matter and in a percentage of the Mn-content of plants grown on the soil richest in manganese

Geological origin of soil	Wheat	Rye	Lucerne	Red clover	%
Andesite soils	73	53	42	57	100
Triassic soils	54	40	44	42	80
Acidic sandy soils	56	39	39	40	73
Loess soils	48	29	38	45	71
Alluvial soils	33	37	30	49	66
Calcereous sandy soils	37	25	33	40	60
Alkali soils	33	20	40	31	55
Fen soils	25	15	36	23	44

According to our investigations the average manganese-contents of the test plants grown on various soil-types are lower than those published in the literature, that is due to the efficient Mn-supply related with the pH of the soil.

The manganese-contents of lucerne collected in the same stage of development from areas with different soil types similarly show that the field crops of Hungary are generally poor in manganese, and this decisively influences the Mn-status in phytophagous animals. The grasses — as mentioned before — are usually rich in Mn (Table 10). The ten grasses examined showed a considerable variation of manganese-content; the average values ranged between 70 and 95 mg/kg dry matter during the vegetation. Within this the rye-grass, the onion couch and the blue grass are poorer, and the dactylis is the richest in

Table 10

Changes in the Mn-contents of grasses from the same place in the course of development and during the vegetation period (mg/kg dry matter)

	Growth I.		Growth II.		Growth III.
	young	after earing	young	older	autumn after-growth
Rye grass	57.3	63.9	—	68.0	69.2
Meadow fescue	81.2	66.9	—	120.2	112.0
Dactylis	115.8	135.3	101.5	134.4	118.9
Hung. brome grass	73.3	61.1	—	123.3	134.2
Tall fescue	59.8	66.1	77.2	91.9	79.4
Reed-grass	63.6	49.8	—	84.9	108.2
Timothy	82.7	94.4	86.8	92.9	91.5
Blue grass	58.2	68.1	—	76.8	79.8
Red fescue	95.3	54.4	100.3	94.0	91.8
Onion couch	60.6	42.5	—	76.4	86.4

Mn. The average Mn-contents of grasses gradually increase growth by growth from the first- to the autumn growth.

The manganese-contents of the indicator plants decreased in the course of development (Table 11) though not in such a high rate as the copper- and

Table 11

Changes in the mangenese-contents of various indicator plants in the course of vegetation (mg/kg dry matter)

		9 April	21 April	4 May	18 May	P
Lucerne	$\bar{x}$	27	30	23	22	>0.05
	s	3	2	2	4	
Red clover from arable land	$\bar{x}$	46	38	38	29	<0.01
	s	3	2	6	3	
Red clover from grassland	$\bar{x}$	—	27	36	23	>0.05
	s	—	4	3	2	
Rye	$\bar{x}$	36	28	18	12	<0.001
	s	2	3	2	4	
Wheat	$\bar{x}$	56	58	42	31	<0.05
	s	8	8	4	4	
Blue grass	$\bar{x}$	59	62	42	26	<0.001
	s	11	6	5	3	

zinc-contents did. Moreover, the Mn-content of the lucerne increased from the first to the second occasion of sampling, and a similar tendency was observed with red clover from grassland, wheat and blue grass.

The manganese-status and the production of animals

The hair shows very well the mangenese-status. In the everyday practice the most readily available material required for the examination of the manganese-status in milking cows is the cover hair, and occasionally the liver-sample taken on salughtering culled animals. The colour and pigmentation of the hair influences its manganese-content; the light or white hair contains significantly less Mn than the dark hair. Besides the colour the seasonal changes must also be taken into consideration. In the period of changing (March–May) the hair contains more manganese than in the other months, therefore this period is unsuitable for manganese diagnoses. The beginning deficiency is indicated by 6 mg/Mn/kg in the black- and 5 mg/Mn/kg in the yellow, red and brown cover hair, per kg dry matter.

In the livers of ruminants 10–12 mg/Mn/kg dry matter is contained on the average.

The manganese-status in the animals was established by determining the Mn-contents of the hair and liver (Table 12). The Mn-supplies of sandy-, alkali-, fen- and loess soils yielding crops poor in manganese were found — in accordance with our expectation — to be deficient; on the basis of the Mn-contents of hair samples the Mn-deficiency of loess soils, the type of soil playing a leading role in crop production — exceeds 80%; similar deficiency was found in the andesite soils; while the alkali soils showed a 50% and the sandy soils a 65% Mn-deficiency. The Mn-contents of the liver samples support these results, since it was found 5.5–6.3 mg Mn/kg dry matter instead of 8 mg, the lower limit of a normal Mn-status.

Table 12

Mn-contents of hair and liver from cows kept on areas with different soil conditions (mg/kg dry matter)

Soil type	Hair		Liver		Deficiency %*
	$\bar{x}$	s	$\bar{x}$	s	
Loess soils	3.3	3.2	5.5	1.2	85
Alkali soils	5.7	2.7	5.8	2.1	50
Sandy soils	5.8	2.5	6.3	0.4	65
Andesite soils	4.5	3.0	5.7	2.1	84

* The deficiency means the percentage of cows containing less than 5 mg Mn/kg dry matter in the hair.

The Mn-supply in the case of free range breeding is good in comparison with indoor stock breeding. Supplements of Mn must be given only exceptionally as supported by our experiments with ewes and meat-type cows. Wild ruminants are also adequately supplied with manganese, though slight Mn-deficiencies may occur in animals living on alluvial- and loess soils.

The mangenese-requirement for ruminants and poultry is 60 mg/kg dry matter of feed. The Mn-requirement for poultry may vary with the breed. Owing to the low manganese-contents of fodders (<60 mg Mn/kg dry matter) a primary deficiency, and in consequence of antagonistic effects (Fe, Ca, P) a secondary one may occur. The Mn-deficiency may cause depression of development, danger of perosis and reduced protrombine activity. In the case of a low Mn-content in the egg the embryo mortality can be high. It is therefore highly important to prepare the layer feed with manganese.

According to the results of experiments the manganese-requirement is lower for pig than for ruminants and poultry. It ranges between 20 and 30 mg/kg dry matter of feed depending on the age of the animal, the intensity of growth, the time of pregnancy and suckling. The Mn-contents of pig fodders generally reach this level.

Secondary Mn-deficiency may occur on feeding high iron-content fodders, e.g. poultry- and particularly pig litter (Anke et al. 1977, 1978; Flachowsky et al. 1976).

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EFFECT OF DEFICIENT CRUDE FIBRE- AND ENERGY SUPPLY ON SOMATIC CELL CONTENT IN PRODUCER'S MILK

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The authors describe the results of experiments carried out in 1985–1986 with 50 cows to determine the rate of increase in the number of somatic cells due to a deficient crude fibre- and energy supply. With mathematico-statistical calculations they have proved that improper feeding leads to an increase in the number of accomplished lactations also influences the somatic cell content of the milk produced.

Keywords: somatic cell content of cow's milk; deficient crude fibre supply; deficient energy supply; mastitest

Introduction

In Hungary the microbiological inspection of the fresh milk was introduced on 1 January 1984. In the following years — in the course of further improvements in the system of milk reception made on the basis of foreign experiences — the somatic cell number of the producer's milk will probably also be taken into consideration as a price factor.

For the last ten years or so, populations of cattle with high genetic production potentials have developed in Hungary, which have a greater demand on maintenance technology and feeding than previously. It is a long-established fact that the mistakes made in maintenance technology lead sooner or later to a decrease in the milk production of animals and to undesirable changes in the milk composition. It was upon this knowledge that our experiments were based.

Materials and methods

In 1985 and 1986 we carried out experiments with 50 cows having different degrees of lactation, but nearly indentical production potentials, to follow the changes in the somatic cell content of the fresh milk. In the experiments a Hungarian spotted × Holstein Frisian R_2 population kept under bound conditions was used.

The results statistically evaluated.

The samples were taken in each case during the morning milking and were presented only by cooling, because they were subjected to analysis within 4 hours following the sampling. For the analysis a "Fossomatic automatic somatic cell counter" was used. Prior to beginning the examinations, mastittests were performed upon each animal to detect possible mastitis cases. The tests were continued throughout the examination period.

In the first year of the experiments the dry matter content of the feed ration contained 15.5%–16.5% crude fibre.

In the second year only 87%–90% of the energy of feed required for their production potential was given.

For the two kinds of feeding we employed the two "Poisson biometry evaluation techniques" using the following formula:

$$\chi^2 = \left(\frac{k_1}{n_1} - \frac{k_2}{n_2}\right)^2 \cdot \frac{n_1 \cdot n_2}{k_1 + k_2}$$

The changes in the somatic cell numbers of the accomplished lactations were evaluated by means of the following formula:

$$\chi^2 = \frac{\sum n_i \left(\frac{k_i}{n_i} - \frac{K}{N}\right)^2}{\frac{K}{N}}$$

Results

In the period of feeding rations deficient in crude fibre, the milk showed after the first 10 days a certain increase in cell numbers which continued from week to week until it ceased in the 3rd week. After 3 weeks the mastitests carried out every week gave uncertain results compared to the first week, while the results obtained after 6 weeks were uncertain and positive in 90% of the animals. According to parallel examinations of the total number of germs the milk produced was first-rate. The data of the three-month experiment are given in Table 1.

Table 1

Changes in the somatic cell number of fresh milk depending on the crude fibre content of the feed ration

Number of lactations	Number of cows		Somatic cell number 10^3/cm		Total cell number 10^3		χ^2
			before	during	$before_{R_1}$	$during_{R_2}$	
	n_1	n_2	the experiments		the experiments		
1	23		30–48	73–149	836	2.405	759.5
2	14		55–84	110–215	975	2.431	622.4
3	10		148–190	215–400	1.664	3.006	385.6
4	4		213–250	410–595	919	1.945	367.5

Error % (α) = 0.1, Degrees of freedom (DF) = 1

Comparative value of trial statistics: 10.8, lower than the calculated χ^2 values, that is the deviations of before- and during the experiments are of a probability of 99.9%

In the second year, during the first 6 weeks of feeding rations with reduced energy content, considerable changes in the number of cells were not observed, however after that period a rapid increase in cell numbers did occur. The weekly mastitests gave uncertain or positive results.

The microbiological quality of the milk produced was first-rate. The results of analyses are shown in Table 2.

A comparison by more Poisson frequencies calculated from the somatic cell numbers present with a feeding method appropriate to the biological requirements, with the accomplished lactations taken into consideration, is shown in Table 3.

Table 2

Changes in the somatic cell number of fresh milk depending on the energy content of the feed ration

Number of lactations	Number of cows		Somatic cell number 10^3/cm		Total cell number 10^3		χ^2
			before	during	$before_{R_1}$	$during_{R_2}$	
	n_1	n_2	the experiments		the experiments		
1	22		25–50	85–157	798	2.534	904.4
2	19		38–77	115–238	1.104	3.396	1.167.3
3	6		115–186	205–390	950	1.740	232.0
4	3		205–245	402–558	665	1.448	290.1

Error % (α) = 0.1, Degrees of freedom (DF) = 1

Comparative value of trial statistics: 10.8, lower than the calculated χ^2 values, that is the deviations of before- and during the experiments are of a probability of 99.9%

Table 3

Comparison by Poisson frequencies of somatic cell numbers present with feeding in conformity with the biological requirements

Number of lactation	Number of cows n_i		Total cell number 10 k_i		χ^2	
	Table 1	Table 2.	Table 1.	Table 2.	Table 1.	Table 2.
1	23	22	836	798	1.468.28	825.11
2	14	19	975	1.104	161.36	106.49
3	10	6	1.664	950	3.812.45	5.503.44
4	4	3	919	665	12.207.41	16.277.90

$N = \sum n_i = 51$, 50 $K = \sum k_i = 4.394$, 3.517

Error % (α) = 0.1 Degrees of freedom (DF) = 3

Comparative value of trial statistics: 16.3, lower than the calculated χ^2 values; thus it is significantly proved that the number of accomplished lactations increased the somatic cell number

Discussion

Conclusions drawn from the results of the experiments:

(1) On the basis of Tables 1, 2 and 3 the probability level of the difference between two Poisson frequencies is proved; the somatic cell number of

the fresh milk produced is a function of both the deficient energy- and crude fibre supply and the number of accomplished lactations.

(2) The trend of the somatic cell number is indicative not only of the possibility of the industrial use of the milk and its prospective consequence, but also of the health conditions of the animals' udders and the conditions of feeding.

(3) The Hungarian standard in effect (MSZ 3698–81) allows maximum 500.000 cells/cm^3. According to our examinations this cell number seems to be high.

(4) Also, the values obtained prove that the production of milk considered inferior for some reason does not by itself give sufficient information on the production potentials of cows.

(5) The somatic cell number of the milk produced gives a quicker response to the deficient crude fibre supply than to the deficient energy supply.

(6) Further investigations are required to reveal the causes of mastities without a so-called microbiological background observed in practice. Namely, some of them might be due to improper feeding.

(7) In the course of breeding the production potential in the new generations is continuously increased. If this is not followed by the optimization of the maintenance and feeding conditions, the animal first tries to produce at the expense of its own body with the final result of damages of health, reproduction biology disorders, abnormal composition of the produced milk and shortening of the useful life.

(8) Owing to the relatively small number of the animals included in the experiments, general conclusions cannot naturally be drawn from the results. However, the tendencies are indicative of the problems which in Hungary — as in other countries — are also raised by the practice.

(9) Further, the results of the experiments suggest that the examination of the somatic cell number and the information of the professional staffs of dairy farms about its results are necessary, since such data may be of great help in their breeding-, keeping-, feeding- and sanitation work.

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RAPID DETERMINATION OF PROTEIN AND FAT CONTENT OF POULTRY MEATS BY SPECTROPHOTOMETRY

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A basic solution has to be made from the examined samples using special chemicals (formic acid, abs. ethanol, methylene chloride and n-propanol) and heat treatment.

The protein determination bases on the UV-light absorption of the solution, which is proportional with the protein quantity. Fat content can be determined by measuring the turbidity by a known quantity of the basic solution developed with formic acid.

Calibration measurements must be carried out only once under the same spectrophotometric conditions.

The accuracies of the methods are similar to the basic methods (Kjeldahl and Soxlet).

The spectrophotometric determinations are rapid, and can be used as control methods of the raw materials, production processes and end products.

Keywords: fat content, poultry meats, protein content, spectrophotometry

Introduction

The quantitative determination of the three chief components (fat, protein and water) of poultry meat is generally very time-consuming. Accordingly it is impossible to determine these components by classical analysis before or during the technological process. The purpose of the present study was to elaborate methods to solve this problem.

The principle of the reported method is the rapid solubilization of the total protein and fat content, followed by spectrophotometry. By determining the quantities of these two components, the water content can be calculated.

The spectrophotometric measurement of the *protein content* is based on the characteristic light absorption of the aromatic amino acids in the examined protein. These aromatic amino acids are tryptophan, tyrosyne and phenylalanine. The light absorption of the protein solution is proportional to the protein content of the sample (Toma and Nakai, 1971; Gábor 1979, 1980, 1983, 1986).

The *fat content* can be determined by measuring the turbidity of the same solution, produced in a special way, which is proportional to the fat content (Nakai and Le, 1970).

Material and methods

The *solubilization* of the sample is carried out in a special flask developed in our laboratory (Fig. 1).

First, 0.5000 g of the sample is heated for 20 minutes with 10.00 cm^3 of formic acid (98%, v/v) at 100 $\pm$ 1 °C in a boiling water-bath.

Fig. 1. Special flask for digestion

A stable suspension is obtained after cooling in tap water. Then, 5.00 cm^3 of absolute ethanol, 5.00 cm^3 of methylene chloride and 5.00 cm^3 of n-propanol are added to it to get the stock solution.

Protein content determination is carried out directly on the stock solution by diluting. 2.00 cm^3 of it with 8.00 cm^3 of ethanol in a glass-stoppered tube (spectrophotometric solution). The optical density of the solution is measured at the wavelength of the absorption maximum, 260 nm.

Fat content determination can also be carried out on the stock solution by diluting, 2.00 cm^3 of it with 8.00 cm^3 of formic acid (98% v/v) in a glass-stoppered tube (spectrophotometric suspension). A turbidity appears which is proportional to the fat content. The reaction time is 20 minutes. The turbidity is measured in the visible region, at 500 nm.

Calibration measurements are necessary to evaluate the measured optical density values. *Kjeldahl* determination was used as the basic method in the case of protein content determination. *Soxhlet* extraction was employed to determine the fat content.

At least four samples with different protein and fat contents have to be prepared. The samples are analyzed by the above-described spectrophotometric and basic methods. (An example can be seen in Table 1).

The regression equations can be calculated from the data by a linear programme as follows:

$$Y = a + b \cdot X$$

where

Y = the measured optical density of the spectrophometric solution,
X = the protein/fat content of the spectrophotometric solution, mg (calculated from the data of the basic methods),
a, b = constants.

Figure 2 shows the calibration line and regression equation for turkey meat.

Protein/fat content calculations. The protein/fat content of the sample can be calculated as follows:

$$P/F\% = X \cdot 12.5$$

where

X = the value calculated from the regression equation,
12.5 = a constant which depends on the weight of the examined sample and on the dilutions carried out for the spectrophotometric measurements.

Table 1

Composition of the calibration samples of turkey breast

No.	Homogenized turkey breast, g	Sea sand, g	Turkey fat, g
1	100.00	0.00	0.00
2	75.00	25.00	0.00
3	50.00	35.00	15.00
4	25.00	50.00	25.00

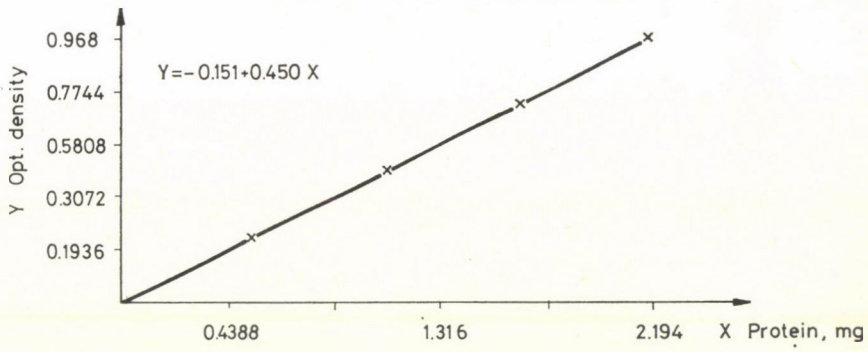

Fig. 2. Calibration line and regression equation of turkey meat

Results

The accuracies of the methods were evaluated statistically. Fifteen parallel measurements were carried out with the basic methods and spectrophotometrically. The correlation coefficients ($r_p = 0.9995$, $r_f = 0.990$) mean that the accuracies of the methods (spectrophotometry and basic methods) are similar.

The calculated statistical parameters were as follows:

t-test:

$$t_{p(\text{calc.})} = 0.45;\ t_{p(\text{table})} = 4.22$$

$$t_{f(\text{calc.})} = 1.02;\ t_{f(\text{table})} = 4.22$$

There are clearly no significant deviations between the average data of the spectrophotometric and basic methods.

F-test

$$F_{p(\text{calc.})} = 1.077;\ F_{p(\text{table})} = 3.7$$

$$F_{f(\text{calc.})} = 2.020;\ F_{f(\text{table})} = 3.7$$

Thus, there are no significant differences between the variance values measured by the spectrophotometric and basic methods.

Discussion

The specific UV absorption values of the protein content determination (optical density values of 0.01 g protein of the examined sample, calculated from the results of *Kjeldahl*-method) varied with the different poultry species. We measured different optical densities for different poultry samples, such as duck, chicken, goose and turkey.

The value of the optical density is practically independent on the different body part of a given poultry species (Fig. 3).

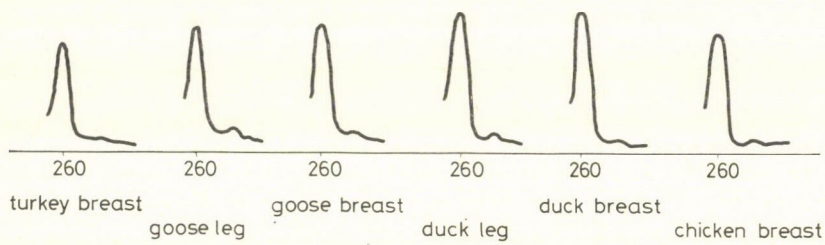

Fig. 3. The spectra of different poultry meats

The spectrophotometric protein and fat content determinations are relatively rapid methods. Therefore, they are suitable for both production control and end-product analysis. In the case of serial examinations, the analysis time for acquiring data is only a few minutes.

For a given species, the calibration measurements have to be carried out only once under constant spectrophotometric conditions.

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Animal breeding

NUTRITIVE VALUE OF SEED MEAL FROM VARIOUS RAPE VARIETIES

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Seed of 14 Hungarian and foreign rape varieties were chemically and biologically analysed. The considerable amounts of crude protein and amino acid contained in the rapeseed meal makes it practical for feeding purposes. The conversion of protein is, however, inhibited by the glucosinolate content. N-metabolism experiments were carried out with rats to determine its biological value, true digestibility, net- and productive conversion, of the protein. Close negative utilisation ($r + 0.879$) was found between the biological value of protein and the glucosinolate content. Of the 14 rape varieties, only "Darmer" and "Tandem" showed low glucosinolate content (4.77 and 4.35 mg/g, respectively) as indicated by the rate of protein conversion.

Keywords: biological value, glucosinolate, nutrition content, rape seed varieties, true digestibility

Introduction

The rapeseed meal left after the extraction of oil should also be reckoned among the protein sources in the feeding practice of Hungary, since the quantities of lysine, methionine, cystine, threonine and tryptophan contained in the extracted rapeseed meal come close to those in the extracted soymeal. Thus, the high protein content and favourable amino acid composition of the former make it suitable for feeding monogastric animals. Nevertheless, its use is limited by a considerable antinutritive effect caused by the glucosinolate content of rapes, except the so-called "00"- or the recently bred "000" varieties. Namely, the rapes contain a glucoside from which under the influence of the myrosinase enzyme thiocyanate (TC) or isothiocyanate (ITC) and L-5-vinyl-2-thiooxazolidone (VTO; goitrin) are produced (Groppel 1983). Their presence in the rapeseed meal above a certain limit causes lack of appetite, hypertrophy of the thyroid gland and depression of growth.

Considering that rape-oil is needed in Hungarian industry, though for human nutrition it has not been used since 1986, the cultivation of rapes is indispensable. The rapeseed meal left after extraction must by all means be taken into consideration as a source of protein.

Rundgren et al. (1985), further Brückner and Mieth (1984) examined rape varieties with different glucosinolate contents. In experiments carried

out with rats they did not find significant differences in the true digestibility of protein, while the biological value and not utilization of protein, and its PER (*p*rotein *e*fficiency *r*atio) value showed great differences depending on the glucosinolate content.

The above justified the comparative trial of 14 Hungarian and foreign rape varieties placed at our disposal by the "National Seed Production and -Marketing Enterprise", Budapest. In the course of our investigations we tried to find an answer to the question of what the effect exercised by the erucic acid and glucosinolate content determined in the various rape varieties on protein conversion in the organism of monogastric animals was.

Materials and methods

Before beginning the chemical and biological analyses we extracted the rape seeds, since they are used for feeding in that form. The nutritive element contents of the extracted seeds of the 14 varieties were determined according to the prescription of the MSZ-6830/65 standard. The amino acid composition was determined for the rapes using a BC-200-type automatic amino acid analyser. The fatty acid composition of the rapeseed oil as well as its glucosinolate content were determined in the laboratory of the Research Institute for Vegetable Oil and Detergent (Budapest). The biological value, digestibility, net- and productive utilization of the protein of rapeseed were established on the basis of N-metabolism examinations with white rats (Szelényi, 1969).

Results

Chemical analyses

The crude nutritive element contents of rapeseed meals deprived of fat are shown in Table 1 with the origin of variety indicated. The data refer to 86% dry matter content. For comparison the data of the extracted Hungarian soybean ISZ-15 are also given in the table.

The samples show considerable differences in crude protein content; that is for 8 rape varieties the crude protein content ranged between 29.9 and 33.7% while for 6 rapes between 34.5 and 38.2%. The latter represent a considerable amount of protein. The crude fibre content of the rapes —9.0–14.5%-exceeds that of the soybean. The latter should be taken into consideration: according to Nehring (1970) in the extracted rape the lignin content may even reach 10%. The ash content varies between 5.5 and 10.0%.

We also determined the amino acid composition for the 14 rape varieties. Out of the essential amino acids lysine, methionine, cystine, threonine and arginine are shown in Table 2, expressed as percentages of the protein- and dry matter content, respectively. The lysine content was 5.6–5.7 g/100 g protein for "Doral", "Savaria", and "Akella", and somewhat more — 6.0–6.4 g/100 g protein — for the other varieties. The methionine content was 1.7–2.0 g/100 g protein, the cystine content 2.0–2.5 g/100 g protein. For comparison's sake we give here the relevant values of the soybean variety ISZ-15: the lysine content

Table 1

Nutritive element contents of various extracted rapeseed meals in 86% dry matter

Variety		Crude protein	Crude fat	Crude fibre	Ash	N-free extr. matter
Name	Origin	percentage content				
Darmor	French	30.7	2.5	11.7	6.3	34.7
Tandem	French	32.5	2.6	12.8	8.4	29.7
Bienvenue	French	29.9	3.4	13.5	6.2	32.9
GK-Savaria	Hungarian	33.7	1.5	12.0	6.0	32.8
Doral	French	33.6	3.2	9.0	10.0	30.1
Claudia	Hungarian	33.2	0.9	14.5	6.1	31.3
Gorczanszki	Polish	35.4	0.9	11.7	6.3	31.7
Emerald	GFR	37.0	1.6	13.7	6.2	27.5
Savaria	Hungarian	34.5	0.9	12.5	6.1	32.0
Akella fresh crop	GFR	38.2	1.2	12.3	5.5	28.8
Petra-Nova	GFR	36.5	0.7	13.1	6.2	29.5
Jet-Neuf	French	36.6	0.7	10.8	6.0	32.0
Borsica	GFR	31.8	0.3	13.6	7.3	32.9
Windal	GFR	33.4	0.8	13.1	6.3	32.4
Soybean (ISZ-15)	Hungarian	39.9	0.5	3.6	7.4	37.1

Table 2

Amino acid composition in various extracted rapeseed meals as percentage of protein- and dry matter content

Rape variety	Protein					%	Dry matter				
	Lys	Meth	Cys	Thre	Arg		Lys	Meth	Cys	Thre	Arg
Darmor	6.1	1.9	2.5	4.4	6.7		1.94	0.60	0.79	1.41	2.14
Tandem	6.2	1.8	2.3	4.6	6.4		2.08	0.60	0.78	1.55	2.15
Bienvenue	6.4	2.0	2.3	4.4	6.5		1.97	0.62	0.71	1.35	2.00
GK-Savaria	6.4	1.8	2.0	4.7	7.0		2.22	0.63	0.68	1.62	2.41
Doral	5.6	1.9	2.1	4.7	6.5		1.98	0.67	0.74	1.67	2.31
Claudia	6.0	1.9	2.1	4.9	6.5		1.97	0.62	0.69	1.62	2.11
Gorczanszki	6.3	2.0	2.1	3.9	6.4		2.21	0.70	0.72	1.63	2.23
Emerald	6.2	1.7	2.2	4.2	6.4		2.45	0.69	0.85	1.65	2.54
Savaria	5.7	2.0	2.2	4.1	6.5		1.92	0.68	0.74	1.37	2.20
Akella	5.7	1.8	2.2	3.6	6.3		2.34	0.75	0.90	1.48	2.56
Petra-Nova	6.1	1.8	2.2	4.3	6.5		2.19	0.65	0.78	1.55	2.33
Jet-Neuf	6.0	1.7	2.1	4.1	6.4		2.33	0.69	0.80	1.60	2.48
Borsica	6.3	2.0	2.3	4.5	6.7		1.94	0.67	0.70	1.40	2.08
Windal	6.1	1.9	2.1	4.9	6.6		1.99	0.60	0.69	1.58	2.13
Soybean (ISZ-15)	6.6	1.6	1.8	3.8			2.65	0.66	0.72	1.55	

is 6.6 g/100 g protein thus exceeding the lysine content of rapes, while the sulphur-containing amino acids show lower values, the methionine content being 1.6, the cystine content 1.8 g/100 g protein.

The fatty acid composition and the glucosinolate content are given in Table 3. for the various rape varieties. According to these data, the varieties "Darmer", "Tandem", "Bienvenue!", "GK-Savaria", "Doral", "Savaria" and "Jet-Neuf" contain minimum quantities of erucic acid in the oil, while in the other varieties this fatty acid is present in considerable amounts (38.7–49.3%).

In Table 4 the isothiocyanates, the 5-vinyl-2-thiooxazolidones and the total glucosinolate contents are seen. In "Darmor" and "Tandem" the total glucosinolate content is 4.77 and 4.35 mg/g, respectively, while in the other rapes it is three or four times greater.

Biological examinations

In N-metabolism experiments with young male white rats we established the major protein conversion indices for the 14 rape varieties and for the soybean used for comparison (Table 5). Accordingly, the biological values of "Darmor" and "Tandem", the varieties with low glucosinolate contents, are 86.3 and 86.8% respectively, higher than that of the soybean examined (81%). With the varieties "GK-Savaria", "Doral" and "Bienvenue", on the other hand, we did not obtain evaluable data owing to the negative N-balance of the animals.

Table 3

Fatty acid composition of oil from various rape varieties %

Rape variety	Fatty acids							
	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:4}$	$C_{22:1}$
Darmor	3.8	0.2	1.0	62.7	20.0	10.5	1.2	0.6
Tandem	4.1	0.3	0.9	58.1	23.1	11.4	1.1	1.0
Bienvenue	4.0	0.2	1.1	62.3	21.1	10.7	0.6	
GK-Savaria	4.2	0.3	0.9	59.7	22.5	10.8	1.6	
Doral	3.7	0.3	1.1	60.9	21.9	11.0	1.1	
Claudia	4.4	0.6	1.5	15.2	13.7	8.8	11.0	44.8
Gorczanszki	4.6	0.8	1.4	12.6	13.2	9.3	8.8	49.3
Emerald	5.5	1.2	1.6	13.4	15.7	8.6	9.4	44.6
Savaria	5.7	1.3	1.7	54.6	22.2	10.0	1.6	1.9
Akella	5.3	1.0	1.7	15.9	16.0	9.1	12.3	38.7
Petra-Nova	5.3	0.9	1.7	13.1	15.3	10.4	9.5	43.8
Jet-Neuf	6.1	1.1	1.8	54.9	22.4	10.9	2.2	0.6
Borsica	3.0	0.2	0.8	13.8	15.4	8.4	9.3	49.1
Windal	3.3	0.3	0.8	13.3	14.7	9.4	9.4	48.8

Table 4

OTC-, VTO**- and total glucosinolates contents in various extracted rapeseed meals* mg/g

Rape variety	ITC	VTO	Total glucosinolate
Darmor	1.33	3.44	4.77
Tandem	0.96	3.39	4.35
Bienvenue	3.67	9.76	13.40
GK-Savaria	3.49	11.20	14.69
Doral	2.72	8.67	11.39
Claudia	4.04	9.68	13.72
Gorczanszki	3.05	10.46	13.51
Emerald	4.49	11.12	15.61
Savaria	2.59	9.87	12.46
Akella	3.38	11.08	14.46
Petra-Nova	2.51	12.73	15.24
Jet-Neuf	3.31	15.23	18.54
Borsica	3.16	9.34	12.50
Windal	3.34	10.48	13.82

* = isothiocyanates
** = 5-vinyl-2-thio-oxazolidones

Table 5

Major protein conversion indices for various rape varieties %

Rape variety	Biological value	Actual digestibility	Net conversion	Productive conversion
	of protein			
Darmor	86.3	82.1	70.9	47.7
Tandem	85.8	82.9	71.1	47.6
Bienvenue	non-evaluable			
GK-Savaria	non-evaluable			
Doral	non-evaluable			
Claudia	49.4	81.8	40.3	17.3
Gorczanszki	51.3	77.5	39.9	16.9
Emerald	50.6	80.3	40.7	17.7
Savaria	48.9	78.3	38.3	15.5
Akella	51.3	82.2	42.3	19.4
Petra-Nova	41.2	79.1	32.6	9.8
Jet-Neuf	47.8	83.8	40.0	17.1
Borsica	48.3	81.5	39.4	16.5
Windal	52.5	83.4	44.0	21.2
Soybean (ISZ-15)	81.0	74.0	60.0	48.0

At the same time, the true digestibility of protein was surprisingly better (77.2–83.8%) than that of the soybean (74.0%).

The net utilization of protein, which expresses how much of the protein consumed was used up, is 60.0% for the soybean, and 71.7 and 70.9% for the varieties "Tandem" and "Darmor", respectively; that is they are essentially better than in the case of soybean. For the other rape varieties the respective values range between 32.0 and 44.0%.

The productive utilization of protein, which expresses the relation of the N-balance and the protein consumed is 47.4 and 47.6% for the rape varieties "Darmor" and "Tandem", and 48.0% — that is practically the same — for the soybean.

For the varieties "Bienvenue", "GK-Savaria" and "Doral" evaluable data could not be obtained, while the other rapes only showed 9.8–21.2% productive protein conversion.

Conclusions

The considerable amount of crude protein and the balanced essential amino acid content found in the extracted rapeseed give reasons for using it in the feed of monogastric animals. These excellent properties of the rape are, however, counteracted by the glucosinolate content as an antinutritive factor. Between the biological value of protein and the glucosinolate content $r = -0.879$ correlation ($y = 96.2–3.25x$) was pointed out, which is confirmed by the examination results of Bille et al. (1983) and Rundgren et al. (1985). At the same time the erucic acid does not cause that kind of effect, as pointed out by Schulz and Petersen (1978) in their experiments on rape. Bell and Jeffers (1976) analysed an average lot of extracted rapeseed containing 36% crude protein and 11% crude fibre beside and 8.5 mg/g glucosinolate content; and they too found that the glucosinolate content had the greatest effect on the nutritive value of the rapeseed meal. As for its effect on digestion biology, this factor precedes the protein-, amino acid- and fibre content.

The examination results offer two possibilities concerning the use of rapeseed meal for feeding purposes:

(1) Only those varieties ought to be introduced in commercial production which through breeding have erucic acid- and glucosinolate contents not exceeding the values established for the varieties "Darmor" and "Tandem" in Table 5 (4.7–4.3 mg/g);

(2) The other possibility is to make the high glucosinolate content rapes suitable for feeding by various treatments. Techniques based on extraction and energetical requirements. Other methods for decomposing damaging compounds, such as e.g. treatments with formaldehyde and calciumhydroxide similarly exclude the use of rapes in feed mixtures (Lüdke et al., 1985).

The method given in (1) is only reasonable if the sowing are of the antinutritive rapes can be reliably isolated from that of the "00" rapes. Namely, experience has shown that seeds with high glucosinolate content left from the previous year's production germinate and these plants develop together with plants grown form "00" seeds; or in the pollination period antinutritive rapes from not too distant rape fields destroy the favourable genetic properties of "00" varieties.

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STEROIDAL GLYCOSIDES AS PLANT RESISTANCE INDUCTORS

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The antiviral activity, the mechanism of action and the practical possibilities for using natural and biologically active substances, namely steroidal glycosides, have been investigated by the "tobacco mosaic virus-tomatoes" model system. It has been found that they can affect the virus itself and the host plant metabolism, changing the protein composition and the cell ribonuclease activity of the host plant. They result in decreased tobacco mosaic virus infectivity and increased host plant resistance. Consequently, steroidal glycosides can be used as plant resistance inducers in agricultural practice. The increase of the total tomato plant resistance and yield by treating seeds with steroidal glycosides averages 40%.

Keywords: steroidal glycosides (SG), SG antiviral activity, SG action, tobacco mosaic virus (TMV), resistance inducers

Introduction

A number of new natural compounds — steroidal glycosides (SG) — have been isolated and identified in the late seventies at the Moldavian SSR Academy of Sciences (Kintia et al. 1979). Experiments on various biological test-objects showed that these substances have a wide biological activity spectrum. Since 1979 they have been tested as antiviral compounds.

There are many substances inhibiting plant viruses in vivo: ribonucleases (Martinova et al. 1969, 1979), antibiotics (Markov, 1980, Misra 1977), vegetable extracts (Verma 1970, Allam et al. 1979), polysaccharides (Kovalenko et al. 1982, Gillaspie et al. 1981), physiologically active substances (Aldwinckle 1975, Zhmurko et al. 1971), phenols (Krylov et al. 1976), fungicides (Tomlinson 1977, Tomlinson et al. 1976), purine and pyrimidine base analogues (Foglein et al. 1979, Schuster 1983). Unfortunately, their practical use was found impossible for some reason or other. We know only two preparations of real agricultural value: imanine (USSR, A. D. Bobyr) and DHT with its derivatives (GDR, G. Schuster). It should be noted that the substances mentioned have been selected empirically: the first one out of 700 (Bobyr 1978) and the other — from 400 (Schuster 1986) compounds with antiviral effect. Of course, it by no means reduces their practical value, but significantly hampers the further approach to the search for new antiphytoviral agents. Hence, we have

made an attempt to find a new way to plant protection against virus diseases based on the ability of any living organism to resist infection. The existence of active pathogen resistance mechanisms in plants is now regarded as indisputable ("Active Defense Mechanisms in Plants" 1983).

The viral infection causes changes in plant metabolism, particularly concerning protein exchange (Gianinazzi et al. 1974, "Proceedings of the Workshop on Pathogenesis-Related (b) Proteins in Plants" 1983) and enzymatic cell activity (Ladygina 1971, Omelchenko et al. 1981). A virus-induced activation of oxidative enzymes comparises with unsaturated lipid peroxidation, resulting in increase of free peroxide radical concentration (Zenkov et al. 1975). Hence, we suggested that free radical reation inhibitors bioantioxidants may be used as plant resistance inducers. SG show antioxidant activity caused by a high mobility of hidrogen atoms in the hemiketal hydroxyl group at a glycone C-22 (Kintia et al. 1982). The above-mentioned allowed to screen of these compounds to examine their potential antiviral activity in plants.

Materials and methods

SG antiviral activity investigations have been carried out on the pathosystem model: TMV — tomato plants. Tomato plants of susceptible variety *Tyeplichny-200* have been grown in greenhouse in vegetative pots filled with a chernozem: (3: 1) mixture. At the seminal leaf stage they were inoculated by TMV 0-strain. The infected plants were cut and put in 0,005% SG water solution at the phase of 5—6 true leaves. Control plants were placed into distilled water. The plants were removed from the solution after 24 hours. The inoculum have been prepared from these plants. Its infectivity being determined from the bioassay on the indicator *Nicotiana glutinosa* L. by the half-leaf technique (Matthews 1973). The TMV infectivity inhibition index has been estimated by Verma's formula (Verma et al. 1970):

$$I = 100\% - \frac{A}{B} \cdot 100\%, \text{ where}$$

I = TMV infectivity inhibition index, %;
A = the number of necroses on the assayed half of the leaf;
B = the number of necroses on the control half of the leaf.

The SG effect was evaluated according to the system of criteria, especially developed for the effect evaluation of the antiviral preparations (Klement et al. 1974). One of glycosides with clear antiviral effect — glycoside 3 — has been used as a model. "*In vitro*" investigations have been carried out on the TMV model. TMV was purified by the known method of PEG sedimentation (Klement et al. 1974).

To investigate the SG action mechanism, changes in protein composition have been defined by the polyacrylamide gel electrophoresis (Davis et al. 1974). The ribonuclease activity was determined on the quantities of acid-soluble products which were generated in the process of the yeast RNA enzymatic hydrolysis (Tatarskaya et al. 1966). The tomato immunization was done by soaking of the seeds in 0.08% SG water solution for 24 hrs. The treated seeds were sown in forming bed and the obtained seedlings were transplanted into the field. When showing massive disease symptoms, plants were evaluated from the level of infection by the diseases of a fungal, bacterial and viral character. The evaluation was performed in accordance with the visual power scale generally adopted in the USSR:

0 — lack of affection
1 point = up to 25% of the vegetative mass is affected
2 points = up to 50% of the vegetative mass is affected
3 points = up to 75% of the vegetative mass is affected
4 points = up to 100% of the vegetative mass is affected

The average infection index was estimated from the formula:

$$X = \frac{a_1n_1 + a_2n_2 + \ldots a_mn_m}{N}, \text{ where,}$$

$a_1, a_2, \ldots, a_m$ = points of plant infection
$n_1, n_2, \ldots, n_m$ = the number of plants with the corresponding infection point
N = the number of plants accounted

Accounts of the plant yielding capacities were taken during the late vegetation development. The experimental data were processed by means of the dispersion analysis (Dospehov 1979).

Results and discussion

The antiviral SG activity has been judged by the TMV inhibition experiment in the tomato plants which were treated with these preparations. In the course of the experiment 12 substances were tested, and all of them showed the ability of reducing the TMV infectivity in tomato plants, i.e. they had a therapeutic effect (Table 1). Consequently, they can be used for the treatment

Table 1

Steroidal glycoside antiviral activity

Glycosides	Average No. of necroses per 1/2 leaves of indicator		Degrees of TMV infectivity inhibition (%)
	control	test	
α-tomatine	75	28	62
Glycoside I	106	68	35
Tomatonine	72	27	62
Tomatoside	89	43	52
Glycoside 3	47	20	58
F-gytonine	81	28	66
Capsycosine	108	46	57
Glycoside 2	73	26	64
Glycoside 4	81	66	19
Digitonin	78	29	63
Glycoside 5	70	62	11
Glycoside 6	44	41	4

of the TMV-infected plants. However, the mechanism of their action must be ascertained first.

This investigation of the SG mechanism of action has been carried out in two stages. During the first one, a general SG action character was studied, i.e. whether they act *in vitro* or *in vivo*, by means of a specially developed criterion system for the evaluation of the virus inhibitor action character (Klement et al. 1974).

Time

The inhibitor affects *in vitro* (on intact virus) and its effectiveness increases as a function of time since the mixing of the inhibitor with a virus-preparation.

The purified TMV preparation was mixed with glycoside 3 and the mixture incubated for 0, 1, 3, 24 hours at the temperature 1–3 °C. At every interval, the mixture was tested on *Nicotiana glutinosa* L. After the appearance of necroses, they were counted and the extent of TMV inhibition was estimated. It was changed in the following way:

(1) At the time of mixing	35%
(2) I hr after the mixing	40%
(3) 3 hrs after the mixing	45%
(4) 24 hrs after the mixing	52%

The results of the experiment show that the effect of the infectivity inhibition of the purified virus, caused by the glycoside 3, gains in strength over time. Hence glycosides, as virus infection inhibitors, may effect *in vitro*.

Dilution

The inhibitor acts *in vitro* if the inhibition effect is retained during dilution.

A mixture of glycoside 3 and a purified TMV preparation was washed out with distilled water, and its infectivity was biotested following dilution. The experimental results, presented in Table 2, show that the glycoside inhibiting effect is also retained under dilutions. It again supports the view that glycosides may act *in vitro*.

Application

The inhibitor acts *in vitro* if the virus infectivity inhibition results only from mixing with the inhibitor before plant inoculation (as in the former two

Table 2

Retention of the SG inhibiting effect during dilution

Dilutions	Average No. of necroses per 1/2 leaves of indicator		Degree of TMV infectivity inhibition (%)
	control	test	
Not diluted	27.50	17.70	36
1 : 1	51.24	29.72	42
1 : 2	10.78	6.63	38
1 : 4	17.07	9.75	43
1 : 8	23.17	13.67	41
1 : 16	7.52	4.74	37

Table 3

TMV infectivity inhibition degree as a function of SG application method

Glycosides	SG are mixed with TMV preparation. The mixture is tested on leaves of an indicator *(in vitro)*			SG water solutions are injected into an indicator plant, which is consequently inoculated with TMV preparation *(in vivo)*		
	Average necrosis No. for 1/2 leaf of the indicator		TMV inhibition degree, %	Average necrosis No. for 1/2 leaf of the indicator		TMV inhibition degree %
	control	test		control	test	
Tomatonine	24	19	−21	12	10	−17
Tomatoside	36	38	+6	14	13	−7
Glycoside 3	32	24	−25	11	7	−36
F-gytonine	17	18	+6	13	12	−8
Capsicosine	—	—	—	11	8	−27
Glycoside 2	—	—	—	12	8	−33

cases). If a plant shows virus infectivity inhibition effect after it was treated with the inhibitor, then the latter acts *in vivo*. Two parallel experiments have been conducted to investigate the SG action pattern according to this criterion. In the first one, the purified TMV preparation was mixed with SG and the mixture was tested on *Nicotiana glutinosa*. In the second experiment, SG water solution was injected into *N. glutinosa* plant, which was then inoculated with a purified TMV preparation. Experimental findings, presented in Table 3, suggest that SG are capable of TMV infectivity inhibition both *in vitro* and *in vivo*.

Thus, the effect of SG as phytoviral inhibitors may be characterized as complex capable not only of reducing the TMV infectivity under direct contact, but also of action in an infected plant. SG seem to influence in a certain way the metabolism of an affected plant, increasing its resistance to viral pathogens. At the second stage of investigation the SG mechanism of action we have examined plant metabolic changes, induced by SG, to confirm the suggestion.

(1) *Protein metabolism.* Virus infection is known to cause protein formations in the leaves of hypersensitive and resistant plants ("Proceedings of the Workshop on Pathogenesis-Related (b) Proteins in Plants" 1983). A similar effect is also observed under the influence of interferon inductors (Gianinazzi et al. 1974). The polyacrylamide gel electrophoresis reveals that glycoside 3 also induces formation of 3 new protein compounds in tomato leaves of the susceptible variety *Tyeplichny 200* : $Rf_1 = 0.24$, $Rf_2 = 0.40$, $Rf_3 = 0.44$. These proteins were not isolated by the authors, and their molecular weight has not been determined, but like other pathogenesis-related proteins, they possibly take part in the protective reactions of plants.

(2) *Ribonuclease activity*. The activity of the ribonuclease, a key cell enzyme, is another biochemical parameter, which changes in response to plant virus infection. A change of that kind might be conditioned by the necessity of protection, directed at suppression of the virus RNA replication (Pantyukhina et al. 1980). In this connection we measured the activity of ribonuclease in tomato leaves, treated with a 0.005% glycoside 3 water solution and infected with TMV. Treatment and inoculation alternations were carried out so as to determine the stage of the infective process, in which the glycoside shows its inhibiting effect.

Experimental scheme

Variants

(1) Control 1 = "pure" plants (not infected with TMV and untreated with SG).
(2) Control 2 = treatment with SG only.
(3) Control 3 = TMV inoculation only.
(4) Treatment with SG 24 hrs before TMV inoculation.
(5) Treatment with SG immediately after the TMV inoculation.
(6) Treatment with SG an hour after the TMV inoculation.
(7) Treatment with SG 2 hrs after the TMV inoculation.
(8) Treatment with SG 3 hrs after the TMV inoculation.
(9) Treatment with SG 24 hrs after the TMV inoculation.

Plants, infected with TMV and treated with glycoside (variants 4–9), were simultaneously tested for infectivity. The infectivity level is shown in Fig. 1c.

A 2.5 times increase of ribonuclease activity, as compared to the control, was found in the course of the experiment both under TMV infection and under SG treatment (Fig. 1b). If the effect is viewed as a cell-protective reaction, then SG is able to induce it. A maximum TMV infectivity reduction (by 48%, variant 5) is observed under a simultaneous glycoside injection and infection, which seems to be caused by the ability of the glycoside to act *in vitro*. Similar effects were also found during the replication cycle of the virus (variants 6–8).

The investigation results of the SG action mechanism give evidence that the compounds in question are capable of inducing protective reactions in tomato plants, which are inoculated with TMV. On these grounds, we tested the SG as plant resistance inducers, or phytoimmunizators, in a field experiment. Seeds have been treated with 0.08% water dilutions of glycosides 1, 2, 3, which showed a maximum effectiveness in preliminary tests. Seedlings, grown from the immunized seeds, have been more vigorous and resistant. Plants flowered and bore fruit earlier. According to the phytopathological evaluation, degrees of infection by a complex of diseases are considerably reduced (Fig. 2),

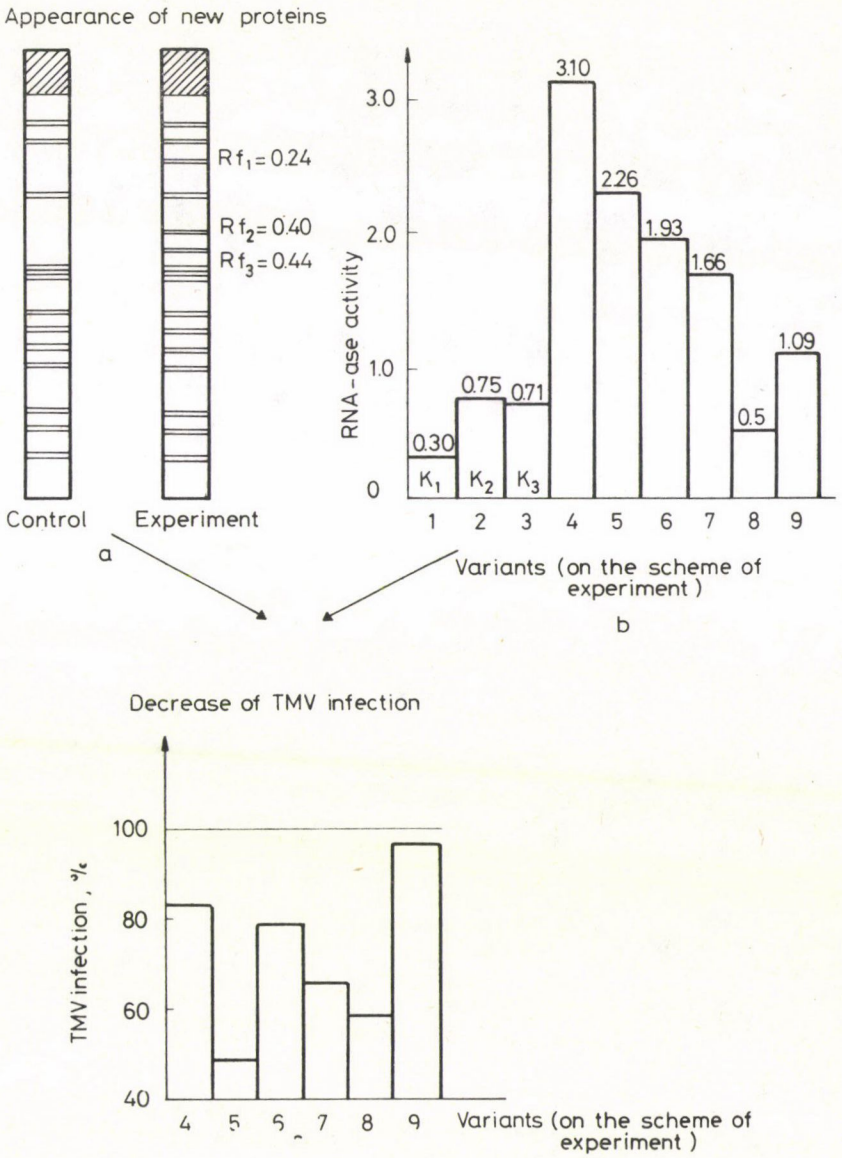

Fig. 1. **Induction of tomato resistance by steroidal glycosides**

which testifies that the total plant resistance increases. While estimating the yield, it was noted that the plant yield increased from 11% up to 41% (Fig. 3). Such an effect is possibly connected with the overall plant resistance increase. Since SG 1, 2, 3 showed their ability to induce protective plant reactions, they may be used in farm management as phytoimmunizators.

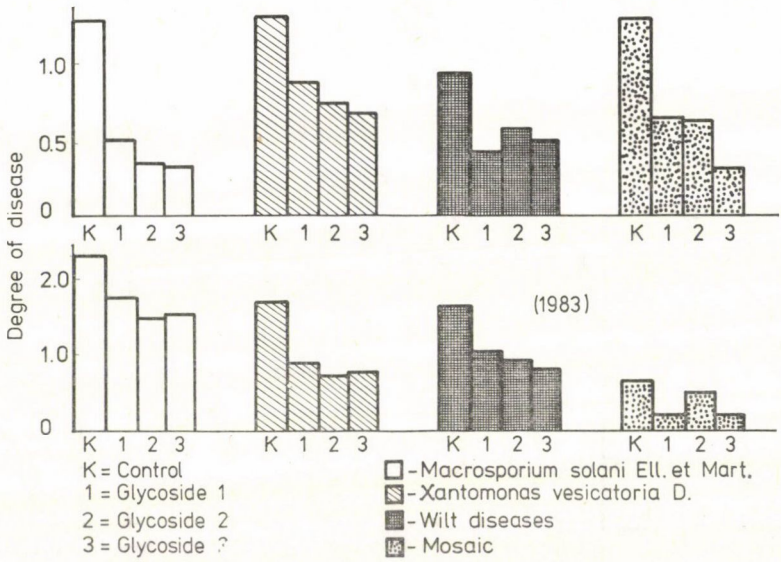

Fig. 2. Decrease of tomato plants infection obtained as a result of immunization of seeds by SG (1982)

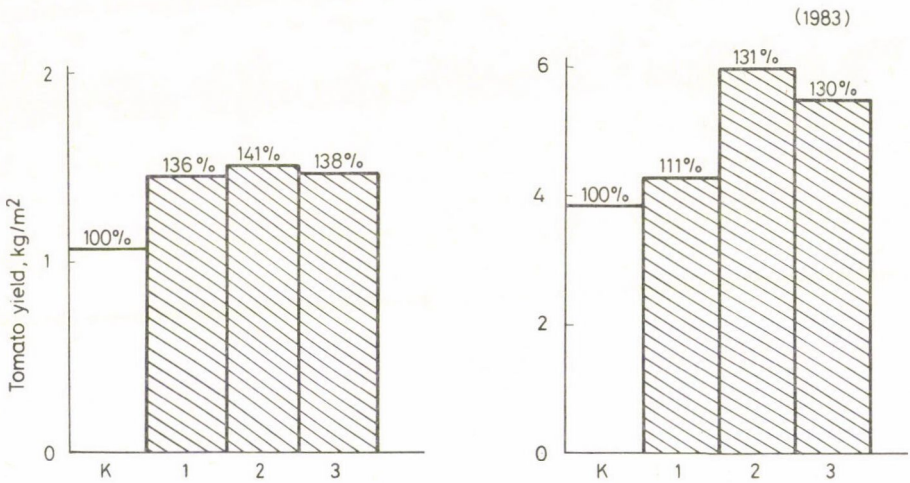

Fig. 3. Increase of tomato plants yield, obtained in result of immunization of seeds by steroidal glycosides (the marks are identical to Fig. 2)

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Book reviews

B. A. AULD, K. M. MENZ and C. A. TISDELL (1987): *Weed Control Economics*. Academic Press Sidney, p. 177.

This Australian book offers detailed information on weed control and its economics. The eight chapters describe the weeds and the methodics of their control, discuss the effect of weed growth on the agricultural production, outline the conomies of weed control taking into consideration the yield loss resulting from the weed cover and the price of the lost yield. This economic threshold is suggested to be used in case the economic efficiency of weed control is the question to be decided. Subsequently, besides extending the use of the threshold the book elaborates models for possible differences in the intensity of weed control. A simple economic threshold model can be widely used in deciding the weed control of field crops. However, the treatment of weed control generally goes beyond such basic models, particularly when the long-term effect of weed control is taken into consideration. The profit of weed control is divided between those applying the weed control and the buyers, because the weed control results in improved quality. Weed control can be correctly evaluated in its social relations only, when not merely the qualitative changes and the market demand of this kind with its effect on expenses, but also the damages to environment are taken into account.

The authors point out that a complex way of control helps to determine the optimum solution. This well-designed book runs to 177 pages with 39 figures, 16 tables and a list of 340 publications. In these days, when the pressing necessity of environment protection and the rising prices of herbicides greatly influence the possibilities of weed control, the book is recommended to those engaged in agricultural economics and plant protection, though practising agriculturists may also make good use of it.

Z. PETRÁNYI

Advances in Soil Science Volume 7. Edited by B. A. STEWART. Springer-Verlag New York, Berlin, Heidelberg, London, Paris, Tokyo.

In accordance with the objective of the series this volume contains five topical subjects from various fields of soil science. It is the work of eight co-authors, three of whom work in Israel, three in Kentucky (USA), one in Canada and one in India.

Part I.: "Modelling of Flow, Transport and Crop Yield in Spatially Variable Fields" was written by ESHEL BRESLER. When adapting results of soil science- and agrochemical experiments to field conditions it is always a problem to apply the results of laboratory, culture pot- and small plot experiments in production. We usually assume that with the points of sampling and the laboratory data obtained the area can be properly characterized and can be regarded as more or less homogeneous. This, of course, is only approximately true. The author wants

to eliminate the errors resulting from the inhomogeneity of the area by applying the stochastic model. For this reason he deals with the infiltration of water into the soil, even giving an example of how to calculate the profile of infiltration in a given case. Detailed mathematical solutions are included for more complicated cases of solution transport too, under given marginal conditions. The model is also applied in yield estimation and economical optimization calculations.

Part II. is D. W. ANDERSON's work: "Pedogenesis in the Grassland and Adjacent Forests of the Great Plains". The exact genetic description of the processes of soil formation provides extremely important and valuable information when the characteristics of given soils are to be interpreted. The author examines the processes important from the standpoint of soil formation, such as the formation and decomposition of organic matter, the relation between carbonate leaching and pH, and the formation and translocation of clay. Special attention is paid to agrochemically important macroelement changes. The genetic description of the area concerned is based on a wide range of data; the solonetz alkali- and forest soils to be found there are characterized each in a subchapter. The author considers the genetic classification of the soil types important, because on this basis, with the knowledge of the processes, the prospective effect of various interventions on the area can be determined.

Part III. M. SCOTT SMITH, WILBUR W. FRYE and JAC J. VARCO in their work: "Legume Winter Cover Crops" consider the various aspects of using legumes as winter cover crops from a scientific point of view. First they compare various known leguminous crops for yield and nitrogen accumulation. Since the cultivation of these crops has an effect on the yield of the main crop and influences the nitrogen turnover and soil properties, these aspects are examined separately. On the basis of the effect exercised on the volume of yield three hypothetical cases are analysed. To examine the N-turnover the fertilizer equivalent is used, and since the key issue is the trend of the amount of available nitrogen which in turn is a function of the decomposition of plant remnants and the N mineralization, it is here that the interpretation of immobilization-mineralization and of the factors influencing this equilibrium becomes necessary. Besides the accumulation of organic carbon and nitrogen, the effect exercised on the moisture conditions (first of all because of the effect of mulch) and temperature of the soil is stressed. As a further advantage the authors point out that, in response to the application of leguminous crops, the physical properties of the soil become more favourable and losses by erosion will also be reduced. Thus, they deem it reasonable to employ this practical method in the future.

Part IV.: "Influence of Sludge Organic Matter on Soil Physical Properties" L. METZGER and B. YARON deal with the effect of sludge. On areas under agricultural cultivation a decrease in organic matter may cause the deterioration of soil, particularly in places where owing to the warm climate the decomposition is rapid, so the application of organic residues and sludges may have a favourable effect. Besides the increase in the nutrient content it may also be the case that the physical properties of soils will improve. The mineralization of various sludge depends on many factors of which the authors mention the major characteristics of the sludge: its source, stability and composition, as well as the parameters of soil, e.g. pH, texture, and such environmental effects as temperature and humidity. They analyse the effect of sludges on the physical properties of soil, primarily on water retention and -conductivity, and on changes in soil structure. As a conclusion the authors establish that properly treated and rationally supplied sludges have a favourable influence on the physical properties of soil and on the organic matter- and nutritive element content, for which their agricultural utilization may be of advantage.

Part V.: "Efficient Resource Management Systems for Drylands of India" was written by J. VENKATESWARLU. Knowledge of the soil- and climatic conditions of the area is

the basis of the correct crop production. In this spirit the local types of soil are shown with profile descriptions based on examination data. The author systematically examines the role of factors determining the yield: the plant, the soil properties, the climatic conditions and the cultivation methods. He evaluates the effects of agrotechnical interventions and analyses the efficiency of fertilization under the given conditions. Special attention is paid to the replacement of organic matter and to the inclusion of leguminous crops in the rotation system. For those areas where the uneven distribution of precipitation is a great problem, the author suggests methods for the elimination of its unfavourable effect. For the utilization of the area concerned a complex system is presented.

The 7th volume of the series Advances in Soil Science, with its 228 pages and 39 illustrations offers valuable help in solving current problems of soil utilization.

MONIKA TAKÁCS

J. SZEGI (Edit.) — *Cellulose decomposition and soil fertility*. Akadémiai Kiadó, Budapest, 1988. 186 pages, 42 tables, 33 figures.

The subject is treated in ten chapters, the first of which deals with cellulose sources and the economic importance of cellulose sources and the economic importance of cellulose decomposition. According to Kovda, the total annual worldwide biomass production amounts to 378 thousand million tons, of which cellulose represents about one-third (126 thousand million tons), and reckoning upon a 44.4% carbon content we may conclude that some 205 thousand million tons of CO_2 are used for cellulose synthesis. This means that a considerable proportion of the cosmic energy is stored in the cellulose molecule.

The second chapter discusses the chemical structure of cellulose and the mechanism of decomposition. Cellulose, a highly polymerized compound built up from 1–4 d glucose, consists of amorphous and micro crystalline parts. Microbiological decomposition greatly depends on the ratio between them. The cellulase enzymes which carry out the decomposition in several steps are at present divided into four groups.

In the third chapter the major groups of cellulose decomposing microorganisms are introduced. The anaerobic cellulose decomposing bacteria, the aerobic bacteria, the actinomycetes and the fungi are described in subchapters.

In the fourth chapter the cellulose decomposing microorganisms are characterized according to the climatic and soil conditions most favourable to them. Thus, under the soil conditions of the tundra the anaerobic bacteria are dominant; leaching and acid pH make the podzol suitable for the multiplication of fungi; in the chemozem the presence of cellulose decomposing bacteria, while in the solonetz soils the appearance of actinomycetes, is characteristic. Of the soil types of Hungary the alkali, the chernozem, the brown forest soil and the sandy soils are discussed here from the standpoint of cellulose decomposing microorganisms prevailing in them.

The next chapter deals with the interaction of microorganisms through their metabolic products. The cellulose decomposing microorganisms, through their metabolic products, create the life conditions for many other microorganisms incapable of making direct use of the carbon content of cellulose. Furthermore, highly important and interesting is the formation of antibiotics and vitamins. The results of international and Hungarian investigations into the above questions are summarized here.

Various environmental effects determine the activity of the cellulose decomposing microorganisms. The optimum temperature is 60—65 °C for the thermophilous and 20–37 °C for the mesophilous cellulose decomposers. The moisture content of the soil is the liquid phase that contains the minerals indispensable for the life of plants and microorganisms. The pH value of the soil often acts not only by changing the H^+-ion concentration, but also as, in the case of acid soils, through

an increase in the amount of soluble aluminium and iron ions. The available sources of nitrogen are decisive for the purpose of cellulose decomposition; the cellulose decomposing bacteria can be divided in groups according to the form of nitrogen that they are able to exploit. The high salt concentration enables, in fact, only the halophytes to take up nutrients from the solution, owing to the high osmotic pressure. For the last two decades, studies on the effect of pesticides on the decomposition of cellulose have come into prominence.

In the seventh chapter the interaction of cellulose, hemicellulose and lignin is discussed. Cellulose is seldom found in its pure state, and usually occurs in combination with hemicellulose and/or lignin. The author presents the results of his experiments to show how these compounds are utilized by the fungi.

The cellulose, when compared to the humns content of the soil, is found to be a much more readily available source of carbon, so it can influence the decomposition of humus either directly or indirectly. This is the subject of the eighth chapter in which humus decomposition in Na-humate by actinomycetes and fungi in the presence of NH_4NO_3 by itself, cellulose by itself, and both together, respectively, are described. A significant difference was observed between the *Actinomycetes* and the fungi; the fungi did not discolour the humus-containing solution without cellulose added to it.

The cellulose decomposition is suitable for determining the biological activity of the soil. In the ninth chapter the author describes three methods for this; first a method based on measuring the quantity of CO_2, then one based on sulphuric acid potassium bichromate oxidation mostly used in model experiments. A detailed account is given of the description and evaluation of the cellulose test. The latter has several variations, which can be equally well used in model experiments, culture pot experiments and in the field. It is demonstrative and with its help the effects of fertilization, liming and irrigation on the biological activity of the soil can be characterized.

In the last chapter which summarizes the Hungarian investigations the effects of fertilization and vegetation are primarily shown, then a highly valuable comparison is made of the cellulose decomposing activity of 25 soil types in Hungary.

The book with its interesting subject and logical structure relies on a wide source of international and Hungarian literature as well as on the author's research results, and makes a valuable contribution to the literature on soil science.

MONIKA TAKÁCS

D. C. MCGEE: *Maize diseases. A reference source for seed technologists.* APS Press, The American Phytopathological Society, St. Paul, Minnesota, 1988. 150 pp.

With the appearance of new diseases in the 1970s and 1980s the phytopathological research activity related to maize increased worldwide, and parallel with it the number of publications dealing with the diseases also increased. Through McGee's book, those phytopathologists who just begin to get acquainted with the diseases of maize, receive great help in their exhausting and time-consuming work of collecting literary data. This book is extremely useful as an enormous collection of data, a wide basis of information which, on the one hand, provides concrete professional knowledge and on the other hand, presents the literary sources related to it. Consequently, it can be used for a long time as a guide for all those who wish to get rapid and reliable orientation in the flow of information on maize diseases.

This book's 150 pages contain essential, indispensable information concerning the various diseases in an extremely concise manner. In the text references are made to the literary sources listed immediately after the description of each disease, not at the end of the chapters nor as an index of the book. The number of the literary sources depends upon the disease discussed: in some cases they are only two or three, while in other cases they may exceed even thirty. A great

value of the book is that the most important literary sources required for further investigations are listed in the same place where the essential information about each disease is given to the reader.

The author classifies the diseases according to the aspects of seed production. *Chapter 1* discusses the seedborne and seed-transmitted diseases. The diseases described in *Chapter 2* occur, though, on or in the seed, but cannot practically be transmitted by seed to the plants. In *Chapter 3* those diseases are included which neither are of seedborne character nor can be transmitted by seed. The diseases described in *Chapter 4* only infect the maize plant through inoculation, i.e. under artificial conditions. Within each chapter the fungal, bacterial and viral diseases form separate groups.

The diseases are described according to a uniform pattern; name of the disease, names of the pathogens, symptoms of the disease, economic importance, geographical distribution, range of hosts, variability of the pathogen, disease control, possibility of spreading by seed, ways of pathogen transmittance, seed dressing techniques, methods of seed examination, and finally a list of references.

As to the description of the diseases, some objections can be made. The author does not distinguish between the current and the synonymous names of the pathogens, but lists them simply one after the other. In the same way, he does not separate the names of the perfect and imperfect forms of fungi. On the other hand, particularly useful is the description of the possibilities of control, where in addition to the chemical control, the biological control methods and control by resistance breeding are also mentioned. Also, what the author says about seed and seed production is highly valuable, e.g. transmission by seed, experimental and practical seed dressing techniques, and the tabulated summarization of methods for seed examination.

To summarize, it can be said that the English literature on maize diseases has been again enriched with an important work. Denis C. McGee's book encompasses all maize diseases known at present. Although according to the sub-title the book was written primarily for those engaged in seed production, we think that it will be recognized as a fundamental work by everyone interested in the diseases of maize, whether they are researchers, teachers or university students.

G. PRINCZINGER

Poljoprivredna znanstvena smotra, Zagreb, 1988. Vol. 53.

The journal is a publication of the Zagreb University, one of the greatest scientific institutions of Croatia, which reports on the research results of the various branches of agriculture: crop production, livestock farming, horticulture and agricultural economics. Most of the scientific papers deal with the agriculture of Croatia, as reflected by the composition of the editorial board, though analyses from other member republics of Yugoslavia are also included in the publications.

The volume is divided in three parts: original scientific papers, reports heard at conferences, and review.
sified concerning their subjects. The results of investigations into soil preservation, agrotechnics, applied plant physiology, phytopathology and reproduction biology provide insights into the research argas of crop production, plant protection, fruit growing and livestock farming.

A. Butorac et al. carried out experiments with lucerne on shallow karst soils. On the basis of the four-year series of tests they established that, in response to liming, positive changes took place in the chemical composition of the soil, and the hay yield of lucerne also increased.

In a three-year experiment M. Cavlek compared the effects of applying nitrogen in nitrate and ammonium forms, on two tobacco varieties. According to the results, the different forms of nitrogen did not cause significant differences in the agronomical features and values of components. The ammonium nitrogen somewhat improved the

quality parameters and increased the yield. Accordingly, the author suggests a higher ammonium proportion and fertilization in stripes.

Sowing time as an important question of oil rape production is discussed in S. Gasperov's paper.

Anatomical and physiological characteristics of the leaves of some fruit species are analysed by K. Dubravec et al. The thickest leaves were found in *Pyrus communis* L., the largest number of stomata per mm^2 in *Juglans regia* L. among the species examined. The highest rate of transpiration with *Pyrus communis* was measured in the final days of August. The authors classify the fruit species examined on the basis of transpiration intensity.

L. Sasuri describes the damage done by four fungi — *Cytospora microspora*, *Nectria galligena*, *Pleospora* infectoria and *Sphaeropsis malorum* in pear and apple. The diseases concerned appear first of all in orchards insufficiently supplied with nutrients, so they can be successfully prevented by proper nutrient management, and with long-action fungicide treatments late in autumn.

The only paper on livestock farming — wirtten by V. Panic et al. — deals with the evaluation of the progeny of intensive milk-type French goat breeds.

B. Palaversic et al. study the effect of nitrogen fertilization on the yield and stalk strength of maize hybrids in various types of soil. Each hybrid responded to increased stand density with a considerable rate of lodging in three soils, except for two cases in fen soil. The hybrid × fertilizer interaction was not significant on any of the soils examined, which shows that the hybrids responded identically to nitrogen fertilization.

The Poljoprivredna Znanstvena Smotra appears in the Croatian language with an English summary and tables. The journal gives an adequate survey of the present work of agricultural research in Yugoslavia. The papers also contain information of practical importance, suitable for immediate use in the everyday life of agriculture.

Z. BEDŐ

The transformation of field crop production in Hungary (1950—1980) M. HAJDÚ (Edit.) Publishing House of the Hungarian Academy of Sciences, Budapest, 1987, 233 pages, 92 charts, 57 illustrations

The authors of the book (I. Dorogi, M. Hajdú, S. Kapás, V. Tibold, Gy. Varga) devoted themselves to an interesting topic, endeavouring to describe the most buoyant period of Hungarian agriculture from many sides. The theme had been perhaps less fruitful, were the investigations not ended with the eighties, and, particularly, if the inferences were drawn.

Notwithstanding these comments, the book is a reliable guide and in some regards also a historical contribution.

Little wonder that the editor had a difficult task handling the diverse material. He surely did his best to co-ordinate the economic, the biological, the technical and the chemical elements, as well as the most important human factors. His efforts were also impeded by the fact that the authors of single chapters tried sometimes to render themselves independent from his general direction. Thus the editor's share had been to eliminate some reiterations and overlaps, in addition to finding a common denominator for different opinions. Although his exertions were not always crowned by perfect success, the goodwill is consequently mirrored by the fair proportions of the text.

The economic aspects of plant production are clearly explained by Varga. Particularly remarkable, and still valid, are his statements about exporting goods of this kind. If in some issues (e.g. as far as the controversial production of soybeans is concerned) his point of view may be debated, on the whole he draws a real picture of trends and interactions of the age.

The chapter dealing with natural resources (Hajdú) is slightly instructive in some parts. Fewer statistical facts would perhaps have been more acceptable. Some of his definitions are, however, rather original, especially those of land fertility and land value.

Kapás compiled the chapter of biological bases correctly, stressing the importance of the subject.

More problematic is that chapter by Dorogi, summarizing the chemicalization of agriculture, which is ambiguously presented. In the opinion he forms of the rapid increase of chemical use during the last three decades, he seems to be a neutral spectator of events. His frequently used expression "soil strength" is not accepted in professional literature. This would, of course, only be a formal defect. What is more to be missed; in summarizing his judgement about fertilizer use and dosing, he does not provide a clear statement (he writes: "the maintenance of soil strength must be dealt with cautious scientifical consideration").

Tibold explains very much in few words within the mechanization chapter. As he is able to fill the gaps of the preliminaries, credit is due to him.

Speaking of human resources Hajdú chiefly examines the educational system. He draws a good comparison between education and production lines. Perhaps he feels most at home in the final chapter, revealing the development of technologies. He boldly discloses his own opinion and his original conceptions. Some overlapping with former parts are inevitable, but otherwise the flow of thoughts might have been broken, which would be regrettable.

In toto the writers' co-operative is praiseworthy, primarily as far as their intentions are concerned. The publisher has also done well; a chronological collection of illustrations increases the value of the volume.

I. DIMÉNY

Advances in soil science Volume 8. (Edited by B. A. STEWART) Springer-Verlag, New York, Berlin, Heidelberg, London, Paris, Tokyo, 1988

The Advances in Soil Science is a series which started to appear in 1985 with the aim of publishing the results of extended research, but mainly state of art review papers, on important actual problems of soil science and related subjects. During the few years of its exstence, the Advances in Soil Science has appeared regularly, two volumes per year, publishing 3–6 papers in each volume. The publications compiled numerous up-to-date questions of soils and their rational utilization as well as methods (both laboratory and filed methods) of investigations.

Volume 8 begins with a Preface by the editor B. A. Stewart, briefly reviewing the actual and future tasks of the series. The editor stresses the international character of the publication as well as its developing popularity in scientific circles.

This Volume consists of four publications:

1. *P. M. Hunag's paper* entitled "Ionic Factors Affecting Aluminium Transformations and the Impact on Soil and Environmental Sciences" describes the new achievements in research related to transformation of aluminium compounds affected by physicochemical agents which is of first-rate importance both in physics and chemistry of soils. The hydrolitic products of aluminium are widely characterized and the problem of clay minerals is also thoroughly discussed. The aluminium, as one of the major elements of the earth's crust, has a detrimental influence not only on soil forming processes, but on the environment as a whole.

The paper summarizes our present knowledge as well as the recent situation in research and methodology.

2. *The second paper* of the volume written by American scientists J. L. Steiner, J. C. Day, R. I. Pependick, R. E. Meyer and A. R. Bertrand is entitled "improving and Sustaining Productivity in Dryland Regions of Developing Countries". The topic of this paper is very topical because of the current food problems of many developing countries with arid and semi-arid areas.

The paper takes a global approach to the problem with a modern definition of dryland conditions as well as with the indication of its geographic situation. The authors pay attention to the decreasing productivity of nearly a hundred countries with dry areas and

analyze the reason of this adverse phenomenon. Physical, chemical, biological constraints of food production are thoroughly described and discussed. Besides of soil factors biological, economical and political concerns are also included.

3. *R. J. Gibbs and J. B. Reid's paper* entitled "A Conceptual Model of Changes in Soil Structure Under Different Cropping Systems" studies both theoretically and practically the problem of changes of soil structures effected by different cropping systems. The problem is rather diverse and very complex, depending on local environmental and farming conditions. The authors do not analyse one or another partial case, but they recommend a rather universal model for study of the process. Both soil factors and environmental factors (meteorological, physical, biological) are included in the model, with great attention to the living materials in the soil.

4. *The last paper* of the volume, written by Indian scientist A. S. P. Murthy entitled "Vertisols of India" deals with a very actual and concrete problem, namely with the vertisols in the Indian subcontinent, where they are very common and typical. The author supplies a detailed physical, chemical and mineralogical characterization of vertisols. His approach is one of the best in the available technical literature.

More than half of the paper discusses the problem of mutrient content and fertility of vertisols, including some recent practical questions of the application of fertilizers. The problem of micronutrients is also discussed.

This Volume 8 of the Advances in Soil Science is a valuable contribution to the modern international activity on soil science.

I. Szabolcs

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INDEX

SOIL SCIENCE AND AGROCHEMISTRY

PLANT PHYSIOLOGY AND BIOCHEMISTRY

PLANT CULTIVATION

PLANT GENETICS

ANIMAL PHYSIOLOGY AND BIOCHEMISTRY

ANIMAL BREEDING

BOOK REWIEWS

SOIL SCIENCE AND AGROCHEMISTRY

PLANT PHYSIOLOGY AND BIOCHEMISTRY

PLANT CULTIVATION

PLANT GENETICS AND BREEDING

ANIMAL PHYSIOLOGY AND ANIMAL BREEDING

CONTENTS

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PLANT CULTIVATION

PLANT GENETICS AND BREEDING

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Agrochemistry and soil science

AVAILABILITY AND CROP UTILISATION OF PHOSPHORUS IN A SALINE-ALKALI SOIL AMENDED WITH INORGANIC AND ORGANIC MATERIALS

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A field experiment involving incorporation of organic materials, viz. *Sesbania aculeata* Pers, FYM, rice husk and poultry manure, and inorganic amendments, viz. gypsum and sulphur alone, as well as conjunctive use of organic materials and inorganic amendments in a calcareous saline-alkali soil, was laid out to study the effect of these treatment on soil amelioration, P uptake, wheat yield and availability of soil P. Results revealed additive effect of organic materials and inorganic amendments in reclaiming the soil and improving the P uptake and yield of wheat. Among different treatment combinations, one involving *Sesbania aculeata* Pers + sulphur provided the best results. The improvements in yield, P uptake and the availability of P in soil was related to the nature and extent of amelioration brought about under different treatments. Stepwise regression analysis show that the uptake of P by wheat could be ascertained by an overall predictability of 95.8% by incorporating the influence of biological index, structural index, pH and EC in addition to capacity, intensity and kinetic parameters.

Keywords: amendments, capacity factor, exchangeable sodium percent (ESP), intensity factor, kinetic factor, mineralisation, reclamation.

Introduction

The solubility and utilisation of native and applied phosphate under saline-sodic conditions has been studied under different experimental conditions with different objectives by several workers (Chabra et al. 1980, Olsen 1953, Poonia et al. 1977, Gupta 1969, Somani 1983). However, the general observations are that the solubility of phosphate increases, remains unaffected or decreases depending upon the nature of the reacting salt, ionic strength and their activity coefficient.

Phosphate availability in the soil arises partly as a result of the continual interplay of mineralization and immobilization by micro-organisms and partly because of release of phosphorus from inorganc phosphorus compounds. It has been reported that phosphorus is mainly taken up by plants

in the form of $H_2PO_4^-$ and HPO_4^{2-}-ions (Hagen et al. 1955, Gupta 1969) whereas PO_4^{3-}-ions constitutes a major fraction of water soluble phosphate under highly sodic situations. Organic manures and inorganic amendments, like gypsum and sulphur, are frequently used in reclamation of saline-alkali soils for improving calcium saturation and soil physical properties. The present investigation was particularly planned to study the availability and utilization of native and added phosphate in a calcareous saline-alkali soil, amended with organic and inorganic materials.

Material and methods

A field trial was conducted on a calcareous saline-alkali soil (typic calciorthids) in Rajasthan, near Udaipur (India). The experiment consisted of fifteen independent treatments involving use of organic materials and inorganic amendments, alone as well as in combination. Four sources of organic materials, namely farmyard manure (FYM), poultry manure (PM), *Sesbania aculeata* Pers. (SA) and rice-husk (RH), were used to supply 0.5% organic carbon in the plough layer (organic carbon added at the rate of 10,000 kg/ha). 50% of the gypsum requirement was met by applying gypsum (6.400 kg/ha) or sulphur (1,195 kg/ha). The fifteen treatment are given below: T_0 control (leaching alone); T_1, gypsum; T_2, sulphur; T_3, FYM; T_4, FYM + gypsum; T_5, FYM + sulphur; T_6, Green manuring of Sesbania aculeata Pers.

Table 1

Physico-chemical characteristics of the soil profile used for the experiment

Characteristics	Horizon			
	A	B	C	D
Depth	0—15	15—30	30—45	45—70
Boundry	Diffuse	Diffuse	Clear	Clear
Colour	10 yr 7/1 light grey	10 yr 5/2 light grey	10 yr 4/1 dark grey	10 yr 3/2 very dark grey
Structure	Blocky	Blocky	Puddled	Puddled
Texture	Clay loam	Clay	Clay	Clay
Consistency	Compact	Compact	Sticky	Very Sticky
pH	9.5	9.3	9.2	9.0
Permeability	Slightly permeable	Less permeable	Impeded	Impeded
Moisture (% dry weight)	3.51	3.83	4.21	4.50
Organic C (%)	0.16	0.14	0.13	0.10
EC (mmhos/cm)	12.81	11.80	10.40	10.10
CEC (me/100 g)	19.55	18.74	17.82	17.16
Mechanical analysis (percentage of mineral matter)				
Sand	55.5	50.6	46.2	44.5
Silt	12.5	12.9	13.4	14.2
Clay	32.3	36.5	40.4	41.3
Exch. Ca (me/100 g)	4.2	4.6	4.2	3.7
Exch. Mg (me/100 g)	8.1	8.1	7.8	7.8
Exch. K (me/100 g)	0.3	0.2	0.2	0.2
Exch. Na (me/100 g)	5.9	5.7	5.4	5.4
$CaCO_3$ (%)	4.2	3.9	5.1	6.8

(SA); T_7, SA + gypsum; T_8, SA + gypsum; T_9, poultry manure (PM); T_{10}, PM + gypsum; T_{11}, PM + sulphur; T_{12}, rice husk (RH); T_{13}, RH + gypsum and T_{14}, RH + sulphur.

The trial was laid out in a randomised block design with above fifteen independent treatments ($t = 15$) and four replications ($r = 4$). For this the land was divided into four blocks of the same size and shape, each block consisting of fifteen treatments. The entire experiment area consisted of 60 plots altogether.

The soil of the experimental field had a pH of 9.4, ESP of 30.23 and EC 12.8 mmhos/cm. The water used for irrigation had an EC of 2.4 mmhos/cm, SAR 13.2 and no RSC. The organic materials used in the study, viz. FYM, Sesbania aculeata Pers, rice-husk and poultry manure, had C : P ratio of 52 : 1, 124 : 1, 249 : 1 and 78 : 1 respectively. A detailed description of soil, irrigation water and of organic materials used in this investigation has been given in Tables 1, 2 and 3.

Table 2

The salinity and other characteristics of the original surface soil and the irrigation water used in reclamation of the area

Characteristics	Saturation extract of soil	Irrigation water
EC mmhos/cm	12.8	2.4
pH	9.4	8.1
Sodium adsorption ratio (SAR)	24.9	13.2
Na^+ (me/l)	82.3	19.1
Ca^{2+} (me/l)	17.5	1.7
Mg^{2+} (me/l)	32.3	2.5
K^+ (me/l)	0.5	0.3
CO_3^{2-} (me/l)	0.7	0.3
HCO_3^- (me/l)	1.2	0.4
Cl^- (me/l)	83.5	16.6
SO_4^{2-} (me/l)	44.5	5.6
Boron (ppm)	0.1	0.1
Fluorine (ppm)	10.2	5.6
Water holding capacity (%)	29.50	
Gypsum requirement (kg/ha)	12800	
Exchangeable Na (%)	30.23	
Hydraulic conductivity (cm/hr)	0.035	
Bulk density (g/cc)	1.84	
Dispersion coefficient (%)	79.05	
Aggregate size distribution:		
Aggregates > 0.25 mm (%)	6.85	
Aggregates 0.10—0.25 mm (%)	10.14	

Sesbania aculeata Pers. was grown as a green manure crop in a neighbouring field in July, 1981. All the organic materials as well as inorganic amendments were incorporated into the soil in September, 1981, in plots 5 × 5 M and the soil kept moist to permit oxidation. Wheat (cultivar: Kalyan Sona) was seeded in the first week of November, 1981 at the rate of 125 kg/ha. The crop was given a balanced fertilizer dose consisting of 100 kg N, 50 kg P_2O_5, 30 kg K_2O and 25 kg $ZnSO_4$ per hectare. The crop was attended by routine agronomic practices and harvested in the third week of March, 1982. Soil samples were collected from the surface 15 cm depth just after harvesting. Wheat grain and straw samples were also collected after harvesting for studying the ameliorative effect of different treatments.

The electrical conductivity (EC), pH, soluble ions, exchangeable cations, $CaCO_3$ content and gypsum requirement (GR) of soil samples were determined using standard procedures described by Richards (1954). Structural Index (SI) (percentage of water stabble aggregates greater than 0.2 mm diameter) was determined using Yoder's (1936) technique. Organic carbon was determined by Walkley and Black (1934) method. Biological Index (BI) (total

Table 3

Chemical composition of organic materials

Constituents	Organic materials			
	Farm yard manure (FYM)	*Sesbania aculeata* (SA)	Rice husk (RH)	Poultry manure (PM)
C %	32.91	48.32	42.33	34.51
N %	1.32	2.68	0.82	1.22
P %	0.63	0.39	0.17	0.44
S %	0.53	0.46	0.15	0.46
K %	0.53	0.86	2.88	0.78
C/N	25 : 1	14 : 1	52 : 1	28 : 1
C/P	52 : 1	124 : 1	249 : 1	78 : 1
C/S	62 : 1	101 : 1	282 : 1	75 : 1

of bacterial and actinomycetes count $\times 10^6$) was determined by the procedures outlined by Allen (1957). The percentile depression in $CaCO_3$ content was calculated using following formula:

$$CaCO_3 \text{ depression } \% = \frac{(CaCO_3 \text{ content of original soil}) - (CaCO_3 \text{ content after harvest})}{CaCO_3 \text{ content of original soil, i.e. prior to start of the experiment}}$$

Equilibrium phosphate potential (EPP) was determined by the method of White and Beckett (1964), Mehta et al. (1954) procedure was used for extraction of organic phosphorus. Olsen et al. (1954) method was employed for estimation of available phosphorus. Dickman and Bray (1940) procedure of colour development was used for determining phosphorus in all the above extracts and in the ternary acid digest of plant material. The ratio of $H_2PO_4^-$: : HPO_4^{2-} present in the soil solution was calculated from the following relationship:

$$\frac{H_2PO_4}{HPO_4} \times 100 = \frac{H}{P^{K_2}} \times 100$$

where P^{K_2} is the second dissociation constant having a values of 6.34×10^{-8} at 25 °C (Bates et al. 1943).

Results and discussion

The effect of incorporating amendments in the saline-alkali soil and their resultant effect on soil amelioration, crop yield and uptake of phosphorus by wheat are described as follows:

Effect on soil properties

The results of physical, chemical and biological properties of saline-alkali soil as influenced by use of organic materials and inorganic amendments followed by leaching, have been presented in Table 4. These data clearly reveal that organic materials or inorganic amendments when used alone, resulted in limited improvement, but they had an additive effect when used together. Sulphur whether used alone or along with organic materials, proved its superiority over similar gypsum treatments. The ratio $Na^+/Ca^{2+} + Mg^{2+}$ decreased from 1.77 in control to 0.30 under treatment involving addition of *Sesbania aculeata* Pers. + sulphur. The soil pH under various treatments

Table 4

Effect of organic materials and inorganic amendments on some physical, chemical and biological properties of calcareous saline-alkali soil under study

Treatments	pH	EC mmhos/cm	Organic C (%)	Exchangeable cations %		Soluble ions		Biological index	Structural index	% $CaCO_3$ depression
				Na^+	Ca^{2+}	$\frac{SO_4^{2-}}{Cl^-}$	$\frac{Na^+}{Ca^{2+}+Mg^{2+}}$			
T_0	9.30	12.55	0.18	26.80	25.49	0.54	1.77	31.5	7.26	1.20
T_1	8.85	10.85	0.21	21.24	31.41	1.03	1.17	37.7	12.09	4.01
T_2	8.80	10.05	0.27	19.49	33.41	1.33	0.92	39.3	14.99	5.22
T_3	9.20	12.15	0.31	24.91	26.51	0.53	1.60	35.1	8.83	2.01
T_4	8.82	10.10	0.39	19.18	33.40	1.34	0.96	40.3	13.82	5.06
T_5	8.70	8.15	0.41	17.66	35.31	2.31	0.51	45.8	16.13	5.86
T_6	8.85	10.40	0.28	21.39	31.61	0.96	1.28	37.2	12.52	4.82
T_7	8.70	9.80	0.30	16.34	36.81	1.39	0.57	45.9	17.93	6.18
T_8	8.40	6.75	0.33	13.75	39.89	4.90	0.30	51.1	23.16	8.43
T_9	9.20	11.80	0.30	24.92	27.60	0.55	1.57	34.7	9.61	2.11
T_{10}	8.75	10.55	0.37	18.39	34.39	1.14	0.96	39.2	15.19	5.62
T_{11}	8.60	7.90	0.41	15.72	36.85	2.87	0.36	44.6	17.42	6.83
T_{12}	9.15	11.65	0.30	24.74	28.13	0.55	1.43	34.1	9.45	2.41
T_{13}	8.80	9.90	0.32	19.71	32.61	1.29	0.83	38.9	14.19	5.25
T_{10}	8.65	7.25	0.38	16.23	35.92	3.80	0.33	43.8	19.53	6.42
SEm ±	0.07	0.54	0.007	0.89	0.86	—	—	1.33	0.34	0.17
CD %	0.20	1.54	0.02	2.55	2.46	—	—	3.81	0.96	0.49

was directly related with exchangeable sodium ($r = 0.98$; $p \leq 0.01$) and inversely with the content of exchangeable calcium ($r = -0.99$; $p \leq 0.01$). The improvement in pH and concentration of soluble salts was found to be associated with corresponding improvements in soil physical properties. Sesbania aculeata whether used alone or along with inorganic amendments (gypsum or sulphur) proved better in favourably improving the physical properties of the soil, salt balance, pH, EC, nutrient availability and crop yield over comparable treatments involving the other organic materials (FYM, poultry manure or rice-husk) because of its succulence, fast-decomposing nature and high content of calcium, besides production of sulphurous acid during its microbial transformation in the soil (Somani and Saxena 1981).

Crop yield and P uptake

The yield and total uptake of phosphorus by wheat (Table 5) increased in direct proportion to the extent of reclamation brought about by the ameliorants under study. The existence of a highly significant value of coefficient of correlation for relationship between P uptake and soil properties like pH, exchangeable sodium percentage, electrical conductivity, biological index,

structural index, percentile $CaCO_3$ depression (Table 6) indicates increased P availability with reclamation. It is, however, interesting to point out that the phosphate concentration in plants growing on control plots was higher, compared with that in plants growing on plots to which organic materials or/and inorganic amendments like gypsum and sulphur were added (Fig. 1) except in plants growing on plots receiving organic materials along with sulphur. A higher nutrient concentration in plants growing on a nutrient-deficient medium could be attributed to restricted plant growth (Paliwal 1972,

Table 5

Yield and P uptake by wheat grown in saline-alkali soil amended with organic and inorganic materials

Treatment	Yield (kg/ha)		% P content		P uptake (kg P/ha)		
	Grain	Straw	Grain	Straw	Grain	Straw	Total
T_0	601	1037	0.581	0.121	3.493	1.255	4.748
T_1	1165	1879	0.533	0.111	6.442	2.086	8.528
T_2	1321	2111	0.557	0.113	7.359	2.386	9.745
T_3	894	1492	0.522	0.110	4.665	1.642	6.307
T_4	1874	2801	0.578	0.118	10.830	3.305	14.135
T_5	2086	3076	0.630	0.130	13.143	3.999	17.142
T_6	1145	1623	0.531	0.109	6.080	1.769	7.849
T_7	2113	3133	0.572	0.115	12.084	3.602	15.686
T_8	2413	3458	0.646	0.133	15.585	4.166	19.751
T_9	800	1429	0.521	0.109	4.168	1.558	5.726
T_{10}	1690	2545	0.601	0.121	10.157	3.079	13.236
T_{11}	1869	2825	0.635	0.128	11.867	3.616	15.483
T_{12}	726	1265	0.513	0.106	3.726	1.341	5.067
T_{13}	1505	2363	0.553	0.112	8.323	2.646	10.969
T_{14}	1700	2659	0.609	0.128	10.353	3.403	13.756
SEm ±	51	56	—	—	0.276	0.057	0.447
CD 5%	146	161	—	—	0.788	0.163	1.277

Table 6

Coefficient of correlation for relationship between P uptake by wheat and parameters of phosphate availability with some soil characteristics

Relationship between	P Uptake	Olsen's P	ESP	EC	EPP	pH
EC	−0.83**	−0.46*	0.92***	—	0.87***	0.92***
pH	−0.82**	−0.56*	0.98***	0.92***	0.98***	—
$CaCO_3$ depression	0.93***	0.56*	−0.98***	−0.92***	−0.88**	−0.99***
Organic C/Organic P	0.28	−0.16	−0.07	−0.12	−0.07	−0.04
EPP	−0.89***	−0.60*	−0.92***	−0.87***	—	0.87***
Olsen's P	0.65**	—	−0.58*	−0.45	−0.58*	−0.56*
ESP	−0.88***	−0.58*	—	0.87***	0.92***	0.98***
Biological index	0.89***	0.68**	−0.95***	−0.92***	−0.92***	−0.93***
Structural index	0.86***	0.53*	−0.97**	−0.51*	0.81**	−0.96***
$H_2PO_4^-/HPO_4^{2-}$	0.90***	0.58*	−0.93***	−0.92***	−0.89***	−0.94***

*** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$

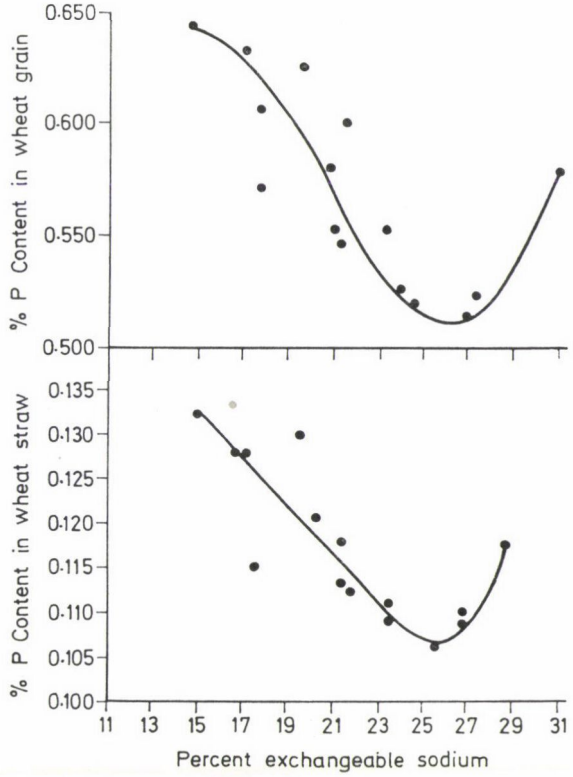

Fig. 1. P content in wheat grain and straw under the reclamative influence of various amendments

Steenberg 1951). The progressive reclamation under various treatments not only improved plant growth but the availability of phosphorus as well (Tables 5 and 7) which resulted in an improved P concentration in the plant.

Soil characteristics in relation to availability and crop removal of phosphorus

(1) Effect of soil pH: The results on soil pH and P uptake show that the pH decreased from 9.30 to 8.40 with progressive reclamation, while total uptake of P increased from 4.748 to 19.751 kg/ha. The uptake of P is significantly related to soil pH ($r = -0.82$; $p \leq 0.001$). An increased P availability with lowering of pH in salt-affected soil was also observed by McGeorge (1939) and Raychaudhari and Landey (1960) which could be attributed to the dominant influence of OH^--ions on the relative proportion of $H_2PO_4^-$ and HPO_4^{2-}-ions in the soil solution (Bates and Acree 1943, Bieleski 1973). Plants absorb most of their phosphorus in the form of primary orthosphosphate ion $H_2PO_4^-$ followed by HPO_4^{2-}-ions (McGeorge 1939, 1932, Russel 1973, Tisdale and

Table 7

Capacity, intensity and rate of release of phosphorus in a saline-alkali soil amended with organic and inorganic materials

Treatments	Total P (kg/ha) after incorporating amendments	Organic P (kg/ha)	Organic C / Organic P	EPP	Olsen's P (kg/ha)	$H_2PO_4^-/HPO_4^{2-}$ (%)
T_0	632	52.2	57.5	7.8	6.4	0.79
T_1	628	56.3	49.7	7.4	10.1	2.23
T_2	631	58.1	44.8	7.2	13.6	2.50
T_3	695	81.3	135.3	7.5	15.1	0.99
T_4	692	101.2	114.6	7.1	19.9	2.39
T_5	693	113.4	104.1	6.7	23.3	3.15
T_6	671	63.3	161.1	7.7	18.4	2.23
T_7	668	75.2	138.3	6.7	22.8	3.15
T_8	670	86.1	123.1	6.3	25.7	6.28
T_9	676	68.3	158.1	7.5	9.7	0.99
T_{10}	672	79.3	141.2	7.2	13.8	2.80
T_{11}	674	89.9	129.2	6.9	17.2	3.96
T_{12}	649	57.8	176.5	7.6	1.5	1.12
T_{13}	646	61.7	168.6	7.1	3.2	2.50
T_{14}	647	66.9	164.4	6.9	5.6	3.53
SEm ±	—	1.82	0.49	0.056	0.49	0.13
CD 5%	—	5.21	1.41	0.16	1.41	0.38

Nelson 1970). According to Hagen and Hopkins (1955) and Sauchelli (1965) plant roots have as many as ten times the absorption sites for $H_2PO_4^-$-ions as for HPO_4^{2-} ions. At each reaction the OH^- ions attract one of the phosphate's hydrogen atoms to form a molecule of water, leaving behind HPO_4^{2-} or even PO_4^{3-} ions. There was a progressive increase in the concentration of OH^- ions, and also the relative proportion of HPO_4^{2-} ions (Fig. 2 and Table 7) within the pH range from 8.4 to 9.3 observed in the present study. The lowered pH with a consequent increase in the concentration of HPO_4^{2-} ions from 0.79% in control to 6.28% in treatment involving addition of *Sesbania aculeata* Pers. along with sulphur, thus resulted in an increased uptake of phosphoruls with progressive reclamation.

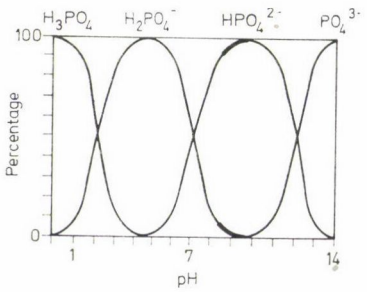

Fig. 2. Relative distribution of different species of phosphate ions at various hydrogen ion concentrations (pH). The darkened portions show the range under investigation

In addition to its influence in controlling the relative proportion of $H_2PO_4^{2-}$ ions, the OH^- ion also competes or interferes with the access of phosphate ions to root cells (Arnon et al. 1942, Overtstreet and Dean 1951). The ability of plants to absorb various ions from the culture medium is apportioned among several different ion groups, and the allotment to each group is shared proportionally by the ions of that group which may be present in the nutrient medium, but not by ions of other groups. Within the group each ion competes with the other for absorption by plants. Plants, possibly treat the $H_2PO_4^-$ ions as members of separate groups, and accordingly, neither is permitted to use any part of the others' entry quota (Bielski 1973, Sauchelli 1965). The OH^- ion competes with both these phosphate ions and thus helps explain why the uptake of phosphate was less with the increased pH of the nutrient medium. It is also possible that the root membrane undergoes a physiological change to decrease the permeability to phosphate more so with increasing OH^- ion concentration as reported by Pratt and Thorne (1948) and Sauchelli (1965).

(2) Effect of $CaCO_3$: The$CaCO_3$ is known to have a depressing effect on the absorption of phosphate by plants, by decreasing the solubility of soil phosphate and its tendency to maintain a high pH (Bieleski 1973, McGeorge 1939, Russel 1973). The initial $CaCO_3$ content of untreated soil (4.15%) was depressed by 1.20% in control to as high as by 8.43% in treatments involving the addition of Sesbania aculeata Pers + sulphur (Table 4). In general application of sulphur greater decrease in $CaCO_3$ content as compared to gypsum or organic materials. This was expected because of limited solubility of gypsum under alkali situation while sulphur was quickly oxidised to sulphuric acid by soil micro-organisms. The sulphuric acid so formed reacts with $CaCO_3$ to form soluble calcium for replacement of exchangeable sodium, which in turn, is removed with leaching water as shown below:

$$2S + 3\,O_2 + 2H_2O \xrightarrow{\text{Thiobacillus thiooxidans}} 2H_2SO_4$$

$$CaCO_3 + H_2SO_4 \rightleftharpoons CaSO_4 + H_2O + CO_2$$

$$CaSO_4 \rightleftharpoons Ca^{2+} + SO_4^{2-}$$

$$Na^+ \text{ clay} + Ca^{2+} \text{ (solution)} \rightleftharpoons Ca^{2+} \text{ clay} + Na^+ \text{ (solution)}$$

Limited depression in $CaCO_3$ content of soil in treatments involving application of organic materials and/or gypsum could be attributed to dissolution of $CaCO_3$ caused by the carbonic acid produced from the CO_2 evolved by microbial decomposition of organic materials, as well as by growing roots. Of all the organic materials under study, one involving the incorporation of *Sesbania aculeata* Pers. caused the highest depression of $CaCO_3$. This is due to sulphurated hydrogen generated during the decomposition of *Sesbania*

aculeata Pers., which reacts with $CaCO_3$ (Mehta 1951) besides having low cell sap pH (4.09). The use of organic materials and inorganic amendments in combination had an additive effect on depressing the content of $CaCO_3$ in the soil.

The uptake of phosphorus increased as the content of $CaCo_3$ decreased, which is evident from the highly significant correlation between P uptake by wheat and percentile depression of $CaCO_3$ in the soil (Fig. 3). Thorne (1946) found that the $CaCO_3$ decreased water soluble phosphate, and the P removal by crops was approximately inversely proportional to the concentration of $CaCO_3$. Hibbard (1935), Ensminger and Larsen (1944) and Smith

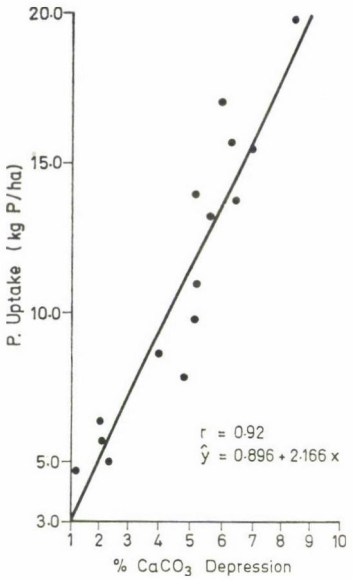

Fig. 3. Relationship between $CaCO_3$ depression in soil and P uptake by wheat

(1948) found that within the range of 1—4% $CaCO_3$ in the soil, the phosphate absorption by plants was inversely related to the $CaCO_3$ content of the soil. The increasing P removal by wheat crops with progressive depression of $CaCO_3$ under various treatments in the present experimental situation is thus fully substantiated in so far as the $CaCO_3$ content was less than 4%. It is also evident from the data presented in Fig. 4 that the $CaCO_3$ depression was significantly related with $H_2PO_4^-$: HPO_4^{2-} ratio ($r = 0.95$; $p \leq 0.01$) which encouraged the preponderance of $H_2PO_4^-$ ions over HPO_4^{2-} ions and helped phosphate absorption by plants.

According to Boischot, Coppent and Hebert (1950) the $CaCO_3$ reacts with monocalcium phosphate and converts it to a less soluble one by increasing

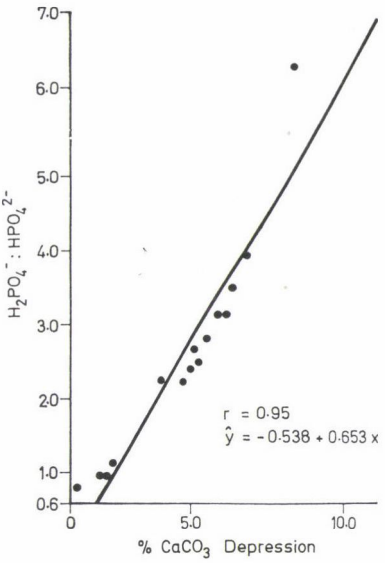

Fig. 4. Relationship between $H_2PO_4^-$: HPO_4^{2-} and $CaCO_3$ depression

the ratio of Ca:P, the $CaCO_3$ furnishing the Ca followed by absorption of excess Ca^{2+} and CO_3^{2-} onto the surface of calcium phosphate crystals. Initially this compound is likely to be bicalcium phosphate but in the presence of $CaCO_3$ it would slowly change to calcium phosphate, richer in calcium and with a lowever solubility of phosphate.

(3) Effect of soluble salts: The data on the concentration of soluble salts as indicated by electrical conductivity has been presented in Table 4. These data show a decrease in the electrical conductivity from 12.55 millimhos/cm in control to 6.75 millimhos/cm in treatment involving the addition of *Sesbania aculeata* Pers. + sulphur. Use of organic materials along with inorganic amendments caused greater lowering in the contents of soluble salts. The phosphate removal by wheat increased in inverse proportion to the content of soluble salts in the soil. This is further supported by a highly significant value of the coefficient of correlation ($r = -0.83$; $P \leq 0.01$) between P uptake and electrical conductivity (Fig. 5). Besides the removal of phosphorus by plants, the indices of phosphate availability including EPP, Olsen's P and organic P content also improved with reclamation. This decreased availability and crop removal of phosphorus with increasing salinity could be attricuted to the interaction of alkali chlorides with the $CaCO_3$ resulting in production of phosphate compounds of lower solubility (Buhehrer 1932). Lehr and Wesemael (1952) also reported decreased phosphate solubility with increasing concentration of neutral salts, the depressing effect increasing in

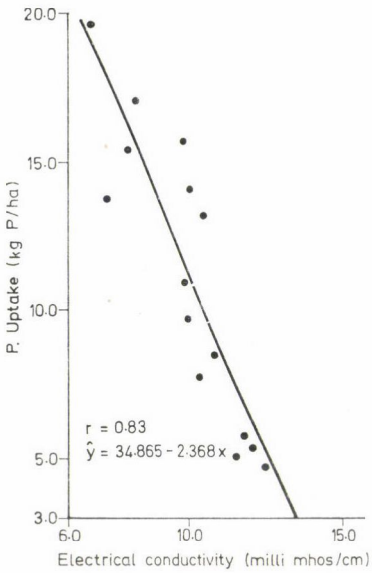

Fig. 5. Relationship between P uptake by wheat and electrical conductivity of soil

the order: Na, K, Mg, Ca and that the effect was less marked with sulphate salts than with chloride salts. Reitemeir (1946) in his studies on the effect of dilution on the soluble salts in several calcareous soils also observed consistently increasing phosphate concentration with increasing dilution. With the same quantity of water applied under various treatments, the dilution of soluble salts thus increased inversely with the concentration of soluble salts left in the soil. The lowest electrical conductivity observed under the treatment involving addition of *Sesbania aculeata* Pers. + sulphur provided more diluted soil solution in this study and resulted in the highest uptake of phosphorus from soil. The observed increases in the concentration of available P with dilution could be attributed to solubilization of insoluble phosphates by additional water, dilution of calcium salts and anion replacement by hydroxyl ions (Somani 1983). The magnitude of *Donnan* distribution of phosphate ions in a calcareous soil will be expected to decrease with increasing concentration of soluble salts, thereby leading to a limited uptake of phosphorus by the plants.

(4) Effect of soluble SO_4^{2-}/Cl^- ratio: The effect of amendments on SO_4^{2-}/Cl^- ratio in soil solution (Table 4) show that it improved from 0.54 in control to as high as 4.90 in the treatment involving incorporation of *Sesbania aculeata* Pers. + sulphur. The uptake of phosphorus by wheat also increased with this ratio which is evident from a highly significant value of the coefficient of correlation for relationship between SO_4^{2-}/Cl^- and P uptake ($r = 0.90$; $p \leq 0.01$). Srivastava, Sharafat and De (1971) also reported increased availa-

bility of P in alkali soil following incorporation of calcium-supplying amendments. In the present investigation, the ratio of SO_4^{2-}/Cl^- increased with progressive reclamation and was significantly related with available P ($r = = 0.51$; $p \leq 0.05$) and also with EPP ($r = -0.83$; $p \leq 0.01$). An improved availability of P in soil is known to reduce the harmful effect of salinity/sodicity (Manchanda and Singh 1982). Apparently, in the present study the uptake of P was reduced with the level of salinity and alkalinity under different treatments. The effect was more severe in the chloride type of salinity as compared with the sulphate type. (Indulkar and More (1984) also reported that chlorides were more harmful than sulphates to the growth and P uptake by plants.

It is interesting to note that the uptake of P by plants as well as the availability of P in soil decreased as the chloride content increased in spite of the application of the same amount of fertilizer P. These results corroborate the findings of Ferguson and Hedlin (1963). Laughlin, Blom and Martin (1971) who reported that both chloride and phosphorus have antagonistic effects. Possibly, the plant growth and the uptake of P in chloride-dominant saline soils could be improved considerably through an increased application of phosphorus. Carbett and Gausman (1960) also pointed out that the P requirement of plants would be more in chloride than under sulphate-dominant saline conditions. Ravikovitch and Yoles (1971) on the other hand observed a substantial decrease in chloride content of both millets and clovers through P application. The results of this experiment points to the need for judicious application of phosphatic fertilizers to minimize the adverse effect of chloride-dominant salinity.

(5) Effect of organic matter content: The effect of the addition of organic materials and inorganic amendments on the resultant organic carbon and biological index of the soil (Table 4) shows a progressively inverse relationship with electrical conductivity and pH of the soil. The availability and uptake of phosphorus also increased with the improving biological index and organic matter accumulation in the soil. This is also evident from the highly significant values of the coefficient of correlation presented in Table 6.

The decreased availability and decreased crop removal of phosphorus in soils with high pH and electrical conductivity may be attributed to the deleterious effect of salinity and alkalinity on the development of micro-organisms (Gupta and Bajpal 1977, James 1959, Sushkina 1956). The data in Tables 5 and 7 shows improved release and crop reeoval of phosphorus under treatments involving the addition of organic matter along with inorganic amendments as compared to their individual applications. This is attributable to the enhanced mineralization of organic phosphorus and the greater solubilization of insoluble phosphates as a result of increased activity by phosphate- dissolving bacteria (Gupta and Bajpal 1977).

Of all the organic materials under study, the rice husk provided the most decreased availability of phosphorus. This is due to low P content of this organic matter (0.17%), which failed to supply phosphorus in excess of microbial needs, particularly during early periods of rapid microbial decomposition. Fuller, Nielsen and Miller (1956), Kaila (1950) and Somani (1983) also reported immobilzation of phosphorus when the P content of added organic material was less than 0.2%. This also explains increased mineralization and availability of phosphorus when FYM, poultry manure and Sesbania aculeata Pers. having a P content greater than 0.2% were added. The extent of phosphate immobilization was higher when rice husk was used alone: possible, because phosphate immobilization increased with the increased content of exchangeable sodium in the soil (Gupta 1969).

The organic materials like *Sesbania aculeata* Pers. FYM and pultry manure caused an increased availability of phosphorus, more so when applied along with gypsum or sulphur. The extent of phosphorus mineralized, however, varied greatly. The investigation of Pinck, Sherman and Allison (1941) and Ghani and Aleem (1943) indicated that in calcareous soil, the amount of $CaCO_3$ present would play an important role in the mineralization of organic phosphorus. The release of $NaHCO_3$ soluble phosphorus was found to vary inversely with the amount of $CaCO_3$ present. The highest availability of phosphorus under treatment involving addition of *Sesbania aculeata* Pers + sulphur, could thus be attributed to lowest content of $CaCO_3$ under this treatment.

The increased availability of phosphorus with the addition and accumulation of levels of organic matter in the soils is not only due to the liberation of phosphorus contained in the organic materials upon their decomposition, particularly when the phosphorus content of the added material is more than 0.2% (Prabhakar et al. 1972), but also due to the solubilizing effect of carbonic acid produced by evolution of CO_2 by decomposing microorganisms, as well as by growing roots (Sen and Bains 1955).

(6) Effect of soil physical condition: if the soils are not friable or not adequately loose, the roots are restricted in their free growth and development. Soil physical condition thus constitutes an important factor in determining whether or not the plant responds to phosphate fertilizers, and can assimilate and utilize its native reserves.

The physical condition of the soil as measured by its structural index (percentage of water-stable aggregates >2 mm) improved considerably from 7.3 in the control to 23.2 in the treatment involving the addition of *Sesbania aculeata* Pers. along with sulphur. The uptake of phosphorus by wheat also increased with improving soil physical conditions. This is further evident from a highly significant value of the coefficient of correlation for relationship between structural index and P uptake ($r = 0.86$; $p \leq 0.01$).

The data in Table 5 show a limited uptake of phosphorus in the control plot (4.748 kg P/ha) compared to that in treatments involving addition of rice husk (5.067 to 13.756 kg P/ha) despite the fact that available P content was higher in the control (6.4 kg P/ha) compared to that in treatments involving addition of rice husk (1.5 to 5.6 kgP/ha). This higher crop removal of P from soils having a lower content of available P could be attributed to improved soil physical conditions when rice husk was added in the soil. This is further evident by the fact that the use of rice husk along with inorganic amendments resulted in a greater improvement in soil physical conditions. The structural index (SI) rose to 9.45, 14.19 and 19.53 by rice husk alone, rice husk + gypsum and rice husk + sulphur respectively, and led to an increased P removal by the crop, more so with improved physical conditions.

The foregoing discussion thus suggests that, whenever plants fail to assimilate and utilize nutrients, the nutrient should be considered as being unavailable, even though the nutrient is in solution or appears to be readily available as revealed from chemical tests (Page and Bodman 1951). In view of this, relying too much on a strictly chemical definition of availability of plant nutrient may be misleading, and it is only through the adoption of a broader definition that a proper understanding and interpretation of plant growth and fertilizer response can be gained under such a salt-affected soil situation.

Relative contribution from capacity, intensity and kinetic factors towards P uptake:

Somani (1983), Mattingly (1965) and Somani (1983) suggested that plant utilization of P is characterized by capacity, intensity and kinetic parameters. In studies based on simple correlation, it is impossible to separate the direct effect of a parameter from the indirect effect caused by its own relationship with another fraction, unless multiple regression is employed (Srivastava and Pathak 1971). It was, therefore, thought desirable to fit the capacity (Olsen's P), intensity (EPP) and kinetic organic C/organic P) parameters into a stepwise multiple regression analysis. The details of this analysis have been presented in Table 8. The kinetic factor which did not have any relationship in simple correlations, made significant contribution when evaluated after separating its direct effect from the indirect effect resulting from its own relationship with other factors. These parameters ranked in the order: EPP > organic C/organic P > available P, i.e. intensity > kinetic > capacity factors in contributing plant utilizable P with an over-all predictability of 88.9%. This predictability increased to 88.9% by considering the influence of EC and pH. A further rise in predictability to the extent of 95.8% was obtainable when the influence of other soil properties, such as biological index (BI) and structural index (SI) of the soil, were taken into account (Table 8).

Table 8

Stepwise regression analysis showing contribution of various parameters of phosphate availability and some soil characteristics towards P uptake by wheat

Independent variables	B	Multiple R	R^2	ΔR^2	Simple r	Overall F***
EPP	−10.137	0.89148	0.79473	0.79473	−0.89***	50.33
Organic C/Organic P	0.039	0.92091	0.84808	0.05335	0.28	33.49
Olsen's P	0.205	0.94300	0.88925	0.04116	0.65**	29.44
(Constant)	75.44					
EPP	−7.384	0.89148	0.79473	0.79473	−0.89***	50.33
Organic C/Organic P	0.037	0.92091	0.84808	0.05335	0.28	33.49
Olsen's P	0.214	0.94300	0.88025	0.04116	0.65**	29.44
EC	−00.546	0.94810	0.89890	0.00965	−0.83**	22.23
pH	−01.278	0.94830	0.89927	0.00037	−0.82**	16.07
(Constant)	72.53					
Biological Index	−02.704	0.89233	0.79626	0.79626	0.89***	50.81
Organic C/Organic P	−00.044	0.92024	0.84684	0.05056	0.28	33.17
Olsen's P	00.543	0.92845	0.86202	0.01513	0.65**	22.91
EPP	−21.767	0.94300	0.88925	0.02723	−0.89***	20.07
EC	−02.371	0.96183	0.92511	0.03587	−0.83**	22.24
ESP	−00.916	0.97872	0.95790	0.03278	−0.88***	30.33
Structural Index	00.170	0.97896	0.95837	0.00047	0.86***	23.02
(Constant)	303.34					

** $p \leq 0.01$; *** $p \leq 0.001$

Summary

A field experiment was carried out to study the availability and crop utilization of native and added phosphorus from a calcareous seline-alkali soil amended with organic materials (FYM, *Sesbania aculeata* Pers., poultry manure and rice husk) alone as well as in conjunction with inorganic amendments (gypsum and sulphur). The yield of wheat crop under different treatments improved in direct proportion to the extent of reclamation. The phosphorus concentration in plants growing in the control plot and the plots amended with organic materials alone, was higher as compared to that in plants grown in plots amended with organic materials along with sulphur. The relative proportion of $H_2PO_4^-$: HPO_4^{2-} improved from 0.79 in control to as high as 6.28 in soils amended with *Sesbania aculeata* Pers. + sulphur. The uptake of phosphorus was negatively related with pH ($f = -0.82$; $p \leq 0.01$) and electrical conductivity ($r = -0.83$; $p \leq 0.01$), but was positively related with the percentile depression of $CaCO_3$ ($r = 0.93$; $p \leq 0.01$), biological index of the soil ($r = 0.89$; $p \leq 0.01$), structural index of the soil ($r = 0.86$; $p \leq 0.01$) and the SO_4^-/Cl^- ratio in soil solution ($r = 0.90$ $p \leq 0.01$).

Efforts were also made to rank and estimate the relative contribution of capacity, intensity and kinetic factors towards P uptake (by employing a stepwise regression analysis) which was found to be in the order: intensity (EPP) > kinetic (organic C) (organic P) > capacity (Olsen's P) factors with an over-all predictability of 88.9%. The predictability of P uptake rose to 95.8% by incorporating the influence of biological index, structural index, pH and electrical conductivity along with capacity, intensity and kinetic factors. The results led us to conclude that the improved P uptake under different treatments was directly related to the improvement in physical, chemical and biological properties of the soil.

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CHEMICAL POOLS OF ZINC AND THE CRITICAL DEFICIENCY LEVEL FOR PREDICTING RESPONSE OF CORN TO ZINC APPLICATION IN ALLUVIUM DERIVED ALKALINE SOILS

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Distribution of Zn in various chemical pools in 31 soils was studied through fractionations. The major portion of Zn remained in complex and organic forms in non-calcareous soils with higher pH. These two forms of Zn constitute as much as 95% of the total chemical pools of Zn studied. Greenhouse studies of these soils were also made to determine the critical deficiency level of Zn for predicting the response of corn to Zn application. Soil application of 5 mg ZN kg^{-1} soil significantly increased the yield. Both graphical and statistical models of Cate and Nelson indicated the critical level to be 0.75 mg kg^{-1} soil DTPA extractable Zn for differentiating the responsive from non-responsive soils. This level gave a predictability value of 84%.

Keywords: Corn, Triticum aestivum, variety 'Ganga-5', zinc content, zinc application, alkaline soils, plant response.

Introduction

Zinc deficiency in the field crops constitutes a major soil fertility problem in many areas of the world, and for that reason application of zinc to various crops has become a common practice. However, only a small fraction of the applied zinc has been found to be utilized by crops, with a large portion of it becoming fixed in the soils in forms less available to the plants (Iyengar and Deb, 1977). Secondly, there have been spectacular responses to applied zinc and about 50% soils of Punjab are deficient in available Zn (Takkar, 1982). The DTPA extraction of soil has been proved a useful method for diagnosing Zn deficiency (Lindsay and Norwell, 1978; Gupta et al., 1981). The critical limits of soil Zn varies with the crop and the soil. These variations are linked to the differential susceptibility of crops to Zn stress. Even for the same crop, critical levels are not identical in different soils. The critical Zn deficiency level for rice and wheat have been determined in India (Singh and Takkar, 1981; Singh and Shukla, 1985). However, information is lacking on the critical level of Zn for corn grown on alluvial derived alkaline soils. The present investigation was, therefore, undertaken to study (1) the different forms of Zn (viz. water soluble, exchangeable, complexed, organically bound

and remaining Zn) present in soils, (2) the response of corn to Zn application in soils with a range of DTPA extractable Zn, (3) the critical limit of DTPA-Zn in these soils.

Materials and methods

Thirty one soil samples (0—15 cm) representing a range in DTPA extractable Zn were collected from the south-eastern region of Punjab state, India. The soils belong to a great group of ustipsamments. Each soil was air dried and ground with a wooden pestle and mortar to pass through a 2 mm sieve. Soil samples were analysed for different fractions of available Zn by the method of Smith and Shoukry (1968). Five g samples of the dried soil were successively leached with various extractants. In each case, 20 ml of the reagent was added to the soil in a 50 ml centrifuge tube. The tube was shaken for 2 hours and then centrifuged for 15 minutes at 10,000 rpm. The liquid was decanted and saved for analysis. Twenty ml of the nex extractant was then added to the soil and the procedure repeated. In order to determine the Zn that was present in the organic fraction, a series of extractions was made and combined. Thus, following copper acetate fractions, the organic matter was destroyed. The soil was transferred to 400 ml beakers with 20 ml water, and then most of the water was removed by gentle heating. Following this, 20 ml of 30% H_2O_2 was added and allowed to react overnight. The reaction was accelerated by heating the beakers in water to about 75 °C. After the reaction was completed, the soil was again transferred to a 50 ml centrifuge tube with deionized water and the extraction repeated with water, ammonium acetate and copper acetate. The Zn was determined in each of these three extractions separately and then combined to determine the total amount of Zn in the organic fraction. The Zn remaining in the residue was removed with an extraction of 1% Na_2 EITA. Besides this ITPA, the extractable Zn from each soil sample was estimated by the method of Lindsay and Norvell (1978). The physico-chemical characteristics of the experimental soils were determined by the standard procedures.

A green house pot culture experiment was conducted with corn, variety Ganga-5, as a test crop. Each pot was filled with 3 kg of soil in a polythene bag and was treated uniformly with a solution to supply 120, 60 and 60 mg kg^{-1} soil elemental N, P and K through ammonium sulphate, potassium dihydrogen phosphate and potassium sulphate, respectively. Zinc was applied at the rate of 0.5 and 10 mg kg^{-1} soil as $ZnSO_4$ $7H_2O$ solution. There were three replicates. Eight seeds were sown in each pot and these were thinned to three after emergence. Deionized water was added to the pots as required to maintain good plant growth. Plants were harvested after 55 days of plant growth, washed in acidified detergent solution and rinsed with deionized water. Samples were first dried in air, then to a constant weight at 60—70 °C in a hot air oven. Dried samples were weighed and ground in a willey mill having stainless steel blades.

One g of ground plant material was digested in a triple acid mixture of HNO_3, H_2SO_4 and $HClO_4$ in the ratio of 9 : 1 : 3. Zinc in the soil and plant extracts was measured by atomic absorption spectrophotometry.

Bray's percent yield was choosen to evaluate the parameters of soil Zn availability and was calculated as:

$$\frac{\text{Yield without Zn}}{\text{Yield at optimum Zn level}} \times 100$$

The critical deficiency level of Zn in soil was determined by the procedure of Cate and Nelson (1965).

Results and discussion

The soils under study were coarse textured, loamy and to sand loam; alkaline in reaction, pH 8.5 to 10.2; medium in organic carbon, 0.6 to 1.02%; non-calcareous, $CaCO_3$ traces to 1.5% adequate in P. 12 to 59 mg kg^{-1} soil

and adequate in K, 89 to 578 mg kg^{-1} soil (Table 1). There was a wide variation in the different forms of Zn extracted by sequential extraction and the Zn extracted by the DTPA method (Table 2). Consequently, the dry matter yield and the percent response of corn to Zn application varied considerably (Table 3).

Table 1

Some characteristics of the experimental soils

Soil No.	Texture	pH	Organic carbon (%)	$CaCO_3$ (%)	P	K
					(mg kg^{-1} soil)	
1	SL*	10.2	0.30	1.5	59	459
2	LS	9.3	0.21	0.6	28	223
3	LS	9.6	0.06	0.5	20	163
4	SL	9.0	0.36	0.3	12	417
5	SL	10.2	0.24	1.0	15	430
6	SL	10.2	0.27	1.4	20	366
7	SL	9.3	0.51	0.5	27	333
8	SL	10.0	0.42	0.8	17	356
9	SL	9.7	0.45	0.8	32	489
10	SL	8.8	0.69	1.3	17	429
11	LS	9.1	0.39	0.9	30	296
12	SL	8.5	0.51	0.3	25	370
13	SL	8.7	0.63	0.5	30	289
14	SL	8.8	0.75	1.1	25	578
15	SL	8.8	0.36	0.3	27	333
16	SL	9.3	0.36	0.3	20	193
17	SL	8.7	0.45	0.3	35	445
18	SL	9.0	0.63	0.8	30	333
19	LS	9.0	0.45	0.4	20	296
20	SL	8.8	0.63	0.7	59	581
21	LS	8.9	0.12	0.6	20	89
22	SL	9.4	0.60	Tr	20	348
23	LS	8.6	0.66	Tr	27	244
24	LS	8.5	0.66	Tr	59	430
25	LS	9.2	0.21	Tr	49	341
26	SL	8.9	0.27	Tr	39	445
27	SL	8.9	0.18	Tr	54	185
28	LS	8.5	0.33	Tr	22	193
29	SL	8.9	1.02	Tr	59	180
30	LS	8.9	0.24	Tr	59	363
31	SL	8.7	0.51	Tr	27	333
Mean		9.1	0.43	0.71	33	339
S.D. (+)		0.5	0.21	0.37	17	116

* SL — Sandy loam, LS — Loamy sand, Tr — Traces

Different forms of Zn in soils

The data indicated that a bulk of the native Zn was in the complexed and organic forms. The amount of Zn in the different fractions was in this order: reductant soluble > organically bound > complexed > exhangeable >

water soluble. Similar findings were reported previously in some Red and Black soils of India (Raja and Iyengar, 1986).

Table 2

Forms of Zn in different soils

Soil No.	Forms of Zn estimated by sequential extraction (mg kg^{-1} soil)					DTPA Zn (mg kg^{-1} soil)
	Water soluble	Exchangeable	Complexed	Organically bound	Remaining Zn	
1	.44	0.16	4.0	4.3	0.24	0.76
2	.28	1.14	36.8	28.6	0.56	0.68
3	.20	1.84	33.6	14.3	0.60	0.92
4	.08	0.48	8.0	4.3	0.44	0.79
5	.36	0.24	8.0	4.1	0.28	0.60
6	.40	1.04	34.4	24.6	0.24	1.00
7	.32	0.28	3.2	4.8	0.48	0.66
8	.36	1.08	27.2	8.4	0.54	0.98
9	.24	0.72	36.8	12.4	0.40	0.48
10	.04	0.88	30.4	12.3	0.52	0.79
11	.16	1.68	42.4	23.6	1.24	0.56
12	.20	1.52	35.4	23.6	0.56	0.48
13	.28	1.20	25.4	11.6	0.84	0.76
14	.32	0.20	11.8	3.3	0.36	0.48
15	.56	1.08	11.2	4.1	1.56	1.00
16	.20	0.80	22.4	7.9	1.24	0.51
17	.16	1.28	18.4	5.1	1.00	0.46
18	.16	0.30	3.2	3.7	1.80	0.22
19	.16	0.48	35.4	13.6	0.36	0.70
20	.30	0.16	3.2	23.4	0.60	0.98
21	.24	2.24	46.4	16.4	0.28	0.90
22	.16	1.14	27.2	13.6	0.40	0.92
23	.16	0.30	4.8	4.2	1.60	0.51
24	.24	1.44	11.2	8.6	2.40	0.48
25	.16	0.88	3.2	3.1	1.80	0.38
26	.40	0.50	4.0	3.6	1.60	0.56
27	.20	0.08	5.6	4.2	0.92	0.40
28	.32	1.12	8.0	3.7	1.20	0.56
29	.20	0.64	6.4	4.3	0.92	0.32
30	.25	0.54	4.0	5.5	0.70	0.29
31	.20	0.68	17.2	6.4	0.28	0.32
Mean	.25	0.84	18.4	9.4	0.84	0.62
S. D. (±)	.11	0.54	13.9	7.2	0.56	0.23

(a) *Water Soluble Zn:* Water soluble Zn varied from 0.04 to 0.56 mg kg^{-1} soil with a mean value of 0.25. Smith and Shoukry (1968) observed low amounts of water soluble Zn (0.8 ppm Zn) in the soil of USA while Raja and Iyengar (1986) reported 0.08 to 0.47 ppm as the water soluble Zn in Karnataka soils having pH 6.1 to 9.0. The low content of water soluble Zn in soils may be attributed to the alkaline nature of soils (pH 8.5 to 10.2) in which Zn forms water insoluble compounds such as Zn dioxide and carbonates. The high content of hydroxyl ions changes the ionic forms of Zn by forming hydroxides

or oxides. The solubility product of Zn soil complexes and carbonate are expected to be very low at the prevailing pH values of the soils. Since the solubility of Zn^{2+} is highly pH dependent, it decreases 100-fold for each unit increase in pH.

(b) *Exchangeable Zn:* The exchangeable Zn content ranged from 0.08 to 2.24 mg kg^{-1} soil with mean value of 0.84 mg kg^{-1} soil. Exchangeable Zn was 3.4 times more than the water soluble. The exchangeable Zn was less in some soils, which may be attributed to high pH, $CaCO_3$ and low organic carbon. At low pH values some Zn may be present on exchange complex of soils, but at high pH values, the level of Zn in solution is so low that very little Zn will be held on the exchange complex. Ammonium acetate extracted a small amount of Zn, as compared to other fractions, except water soluble Zn, Ammonium (NH_4^+) as ammonium acetate was not able to replace all the adsorbed Zn; therefore, it gives low results.

(c) *Complexed Zn:* The complexed forms of Zn constitute an important source of Zn for plant growth (Viets, 1962). The complexed Zn is held in the soil by weak organic bonding. It can be removed by such weak complexing agents as copper acetate, which would remove from the soil heavy metals, such as Zn or copper that were held in by Chelate-type bonds of a weaker nature than those formed with complexing reagents. Copper acetate extracted more Zn than did the water soluble and exchangeable, and it ranged from 3.2 to 46.4 mg kg^{-1} in the soils. This is attributed to the fact that the exchange reactions can easily go to completion in the soil in the presence of solutions of complexing reagents. Moreover, the precipitated Zn and organically bound Zn also appear in solution as soluble Zn complex, which reduces the concentration of Zn in solution, thereby permitting more Zn to come to solution. The higher extraction power of $Cu(OAc)_2$ may be due to its ability to absorb more strongly bound Zn forms both from organic ligands and exchange sites. These results are agree with those of Brown et al. (1971) and Raja and Iyengar (1986).

(d) *Organic Zn:* The organic Zn is different from loosly held Zn in organic *ligands* (Complexed Zn). This fraction of Zn can be removed only after the organic matter is destroyed (Smith and Shoukry, 1968). Organic Zn varied from 3.1 to 28.1 mg kg^{-1} in soils next to complexed Zn. It constitutes about 31% to the total Zn.

(e) *Remaining Zn:* This form of Zn varied from 0.24 to 2.40 mg kg^{-1} in soils with a mean value of 0.84. The soils high in organic matter, but low in $CaCO_3$ and pH values, have a greater amount of this fraction of Zn and vice versa.

(f) *DTPA extractable Zn:* It extracted the complexed form of Zn in soils and represents the plant-available Zn form. It varied from 0.22 to 1.00 mg Zn kg^{-1} soil with a mean value of 0.62.

Thus the soils generally had a negligible quantity of native Zn as water soluble and a very low amount in exchangeable forms. There was a considerable amount of complexed and organically bound Zn in these soil samples. These results suggested that the complexed form is the major source of plant-available Zn in soil, and zinc strongly bound by organic matter is made available through complex formation.

Soil critical deficiency level and response to Zn:

Critical deficiency level is that which separates the responsive and unresponsive soils, or that concentration below which deficiency occurs. The basic fertility of soil can be judged by knowing its critical level. In the present study the critical deficiency level of DTPA-Zn was estimated for corn. The method described by Cate and Nelson (1965) was used to determine the critical deficiency level of soil Zn. The method consists of plotting Bray's percent yield against soil Zn. A cross is placed over the data and moved until the upper left and lower right quadrants have a minimum number of points (Fig. 1). The critical deficiency value is read from the X-axis where the cross intercepts it. This value can also be computed using the statistical model of Cate and Nelson (1971).

Both these approaches gave the same critical value of 0.75 mg kg^{-1} soil (Fig. 1) for predicting the response of corn to Zn. The probability of the percentage response of corn to Zn application in soil testing, less than the critical value of 0.75 mg kg^{-1} soil, was 84% (Table 3). Several workers have

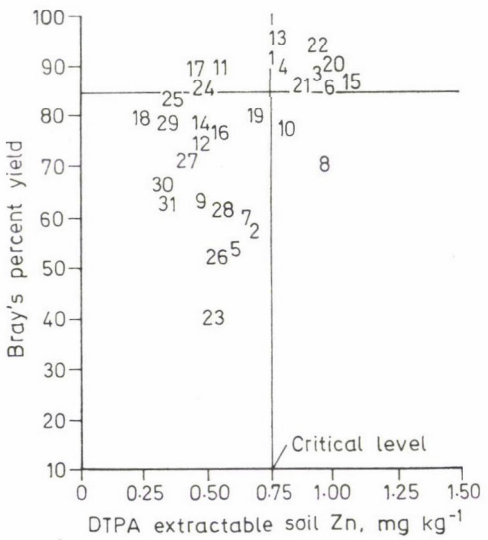

Fig. 1. Critical level of Zn in soil (Numbers indicate the soils as shown in Table 2)

Table 3

Effect of Zn application on the yield and Zn content in corn. Results are mean value of all soil treatment

Parameters	Rates of Zn application (mg kg^{-1})			LSD at P<0.05	Yield increase		% soil responding	
	0	5	10		Deficient soil (≤ 75)	Sufficient soil (> 75)	Deficient soil	Sufficient soil
Yield, g per pot	(16.5) 1.6—27.0	(20.8) 2.4—30.4	(22.3) 2.9—30.8	0.67 —	(28) 4—60	(13) 4—29	85	18
Zn content, mg kg^{-1}	(9.7) 4.5—19.0	(22.5) 13.0—36.7	(32.4) 18.2—58.2	1.7 —				
Zn uptake mg per pot	(0.16) 0.02—0.33	(0.45) 0.04—0.69	(0.69) 0.11—1.28	0.03 —				

Figures in parenthesis are the mean values

reported different critical levels of Zn in soils and crops. Singh et al. (1980) reported 0.75 mg kg^{-1} DTPA-Zn as the critical level for corn in calcareous soils of Bihar and Sakal et al. (1984) suggested 0.76 mg Zn kg^{-1} soil for rice crops in Sub-Himalayan hill and forest soils of Bihar. Sharma et al. (1986) reported 0.55 mg kg^{-1} DTPA extractable Zn as the critical level for rice in Ochrepts, Fluvents and Usterts soils of Hadhya Pradesh.

Corn responded significantly to Zn application. The dry matter yield in the absence of applied Zn ranged from 1.6 to 27.0 g per pot as compared with 2.4 to 30.8 g per pot in Zn treated pots. The percent increase in dry matter yield over control in different soils varied from 4 to 60 with a mean value of 28; while the increase in Zn-sufficient soils ranged from 4 to 29 with a mean of only 13%. Seventeen of the 20 deficient soils responded significantly to Zn fertilization and, of the 11 soils sufficient in Zn, only 2 responded to Zn (Table 3). Zn application sharply increased the plant Zn content; threefold on the average with the highest rate of Zn application. The range of Zn content in plants grown on deficient soils was 4.5 to 19.0 mg kg^{-1} and this increased to 13.0 to 58.2 mg kg^{-1} in Zn treated soils. Consequently, the Zn-uptake by plants also increased significantly with Zn application.

The present study suggest 0.75 mg kg^{-1} soil DTPA extractable Zn as the critical value for differentiating the responsive and unresponsive soils. This method has been adopted as an index of available Zn in many places (Lindsay and Norwell, 1978; Bansal et al., 1980).

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EVALUATION OF SOIL POTASSIUM SUPPLY USING A METHOD OF BIOLOGICAL TESTING

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The potassium uptake of perennial ryegrass (*Lolium perenne* L.) was studied on several soil types at different potassium content (AL-soluble K_2O, ppm). These soils belong to a long-term fertilization experiment representing different levels of potassium supply. The biological test, using ryegrass, was carried out according to the *Chaminade* method. As the consequence of fertilization during 16 years, the considerable differences in the K levels of soils formed two main groups. In the first group, the effect of the different K status did not prove to be regular, while in the second group of soils the potassium uptake of ryegrass showed some relationships with the AL-K_2O levels of the treatments. The highest K uptake in the plants could be measured generally in those treatments, where the AL-soluble K in the soil had also markedly increased. However, this effect was not always accompanied with an increase in dry matter production. The K treatments especially increased the number of the possible cuts of ryegrass. In the treatments without K, plants usually continued growing up to the fifth cut on most of the soils. There were no considerable differences observed between the annually applied potassium doses of 100 and 200 kg/ha, neither in the dry matter production nor in the K_2O uptake of plants. The biological testing method using ryegrass gave sufficient information on the K supplying capacity of soils.

Keywords: biological testing, ryegrass, soil potassium supply.

Introduction

Balanced potassium supply of plants can only be ensured on different soil types where the nutrient requirement necessary for the planned yield is determined with an adequate knowledge of the potassium-supplying capacity of the soil and the particular potassium nutrition of the given plant. This question has been studied by several authors so far (Grimme 1974, Amberger et al. 1974, Sinclair 1982, Todorcic 1974, Schroeder 1971).

In supplying plants with potassium, it should also be considered that even when applying the same fertilizer dose — depending on soil properties — very different amounts of available K can occur in the soil (Németh 1975). According to Jankovic and Németh (1974) surplus yields can only be expected when potassium concentration of the soil solution is increased as the effect of K fertilization.

Grimme et al. (1971) in an experiment carried out with soils containing different levels of exchangeable K, established that the K supply of plants,

and thus yields, is not only a question concerning the exchangeable K content in the soil but also the question of those soil properties which determine the water content of the soil, such as clay mineral content or certain climatic factors. Therefore, it is important to know the characteristics of our soils in the interest of optimizing potassium fertilization, to ensure the adequate nutrient supply to plants.

For the better understanding of these problems, the dry matter production and potassium uptake of ryegrass (*Lolium perenne* L.) were studied in pot experiments with different soil types of Hungary.

Material and methods

The soils originated from 9 locations of a long-term (16 years) fertilization experiment representing different levels of potassium supply (Country-wide Standard Fertilization Experiment) (Table 1).

Table 1

AL-K_2O content (mg/kg) of the experimental soils (0—20 cm) measured at the setting of the pot experiment

Location	Label	K_A+	O	NP	NPK_1	NPK_2
Putnok	PK	42	217	175	296	318
Kompolt	KT	43	166	187	264	304
Iregszemcse	IR	41	156	185	215	359
Bicsérd	BD	43	174	167	251	364
Nagyhörcsök	NK	39	137	151	209	283
Keszthely	KE	36	164	170	263	336
Karcag	KG	47	170	180	259	485
Hajdúböszörmény	HB	58	165	123	195	183
Mosonmagyaróvár	MO	41	215	197	193	207

+ = index of density according to Arany

Treatments applied in the experiment were as follows:

Control — not fertilized for 16 years
NP — annually fertilized with 250 kg/ha N and 15 kg/ha P_2O_5
NPK_1 — annually fert. 250 kg/ha N, 200 kg/ha P_2O_5, 100 kg/ha K_2O
NPK_2 — annually fert. 250 kg/ha N, 200 kg/ha P_2O_5, 200 kg/ha K_2O

Soils in the experiment

Label	Location	Type
PK	Putnok	weakly acid chrenozem brown forest soil
KT	Kompolt	chernozem brown forest soil
IR	Iregszemcse	carbonated chernozem soil
BD	Bicsérd	chernozem brown forest soil
NK	Nagyhörcsök	carbonated chernozem soil
KE	Keszthely	Ramann's brown forest soil
KG	Karcag	meadow chernozem soil
HB	Hajdúböszörmény	meadow chernozem soil
Mo	Mosonmagyaróvár	carbonated fluvisoil of Danube

The experiment was carried out in pots containing 1 kg of soil in four replicates, using ryegrass as a test plant, according to the Chaminade method. In each pot 1000 seeds were sown. Moistening was made to reach 60% of the water capacity in soils. At the beginning of the experiment, AL-soluble (ammonium lactate, pH = 3.7) K_2O content and pH of soils (in KCl extract) were determined. The ryegrass was cut 7 times and dry matter production as well as K_2O uptake of plants were determined.

Results obtained were processed by using a Commodore 128 computer.

Results

The agrochemical data of experimental soils were determined before starting with the experiment. These data are shown in Table 1. Dry matter production, g/pot and amounts of K_2O taken up by plants K_2O mg/pot, are represented in Figs. 1—7.

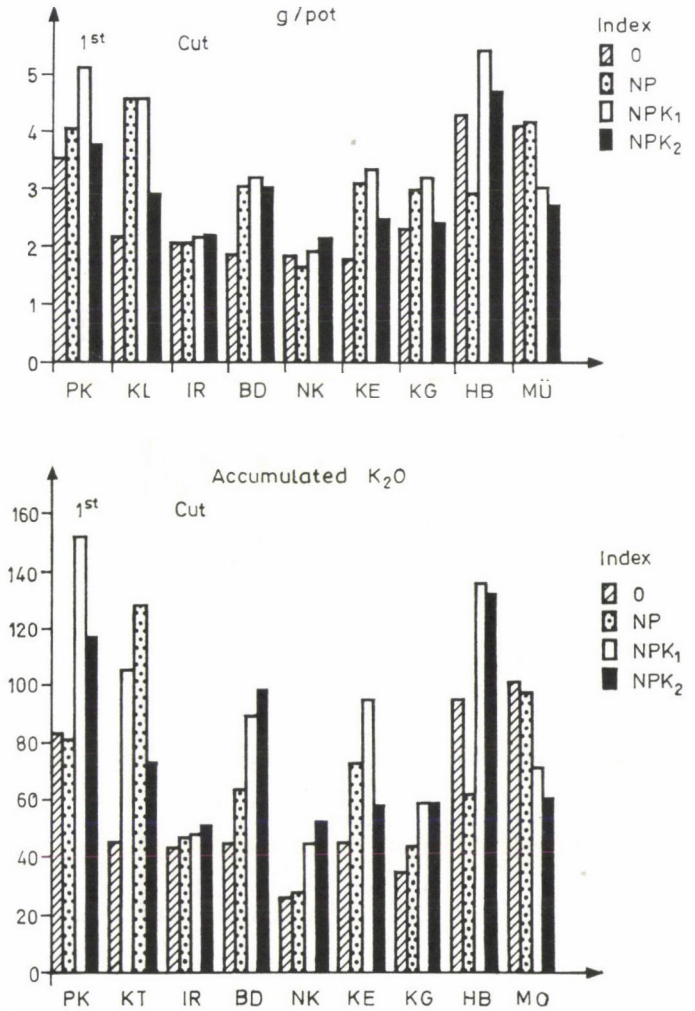


Fig. 1. Dry matter production of *Lolium perenne* and potassium uptake mg/pot

Based on the dry matter data of the unfertilized control, the natural nutrient supplying capacity of various soils can be compared. Results obtained in the 7 cuts reveal an interesting pattern. The dry matter production and

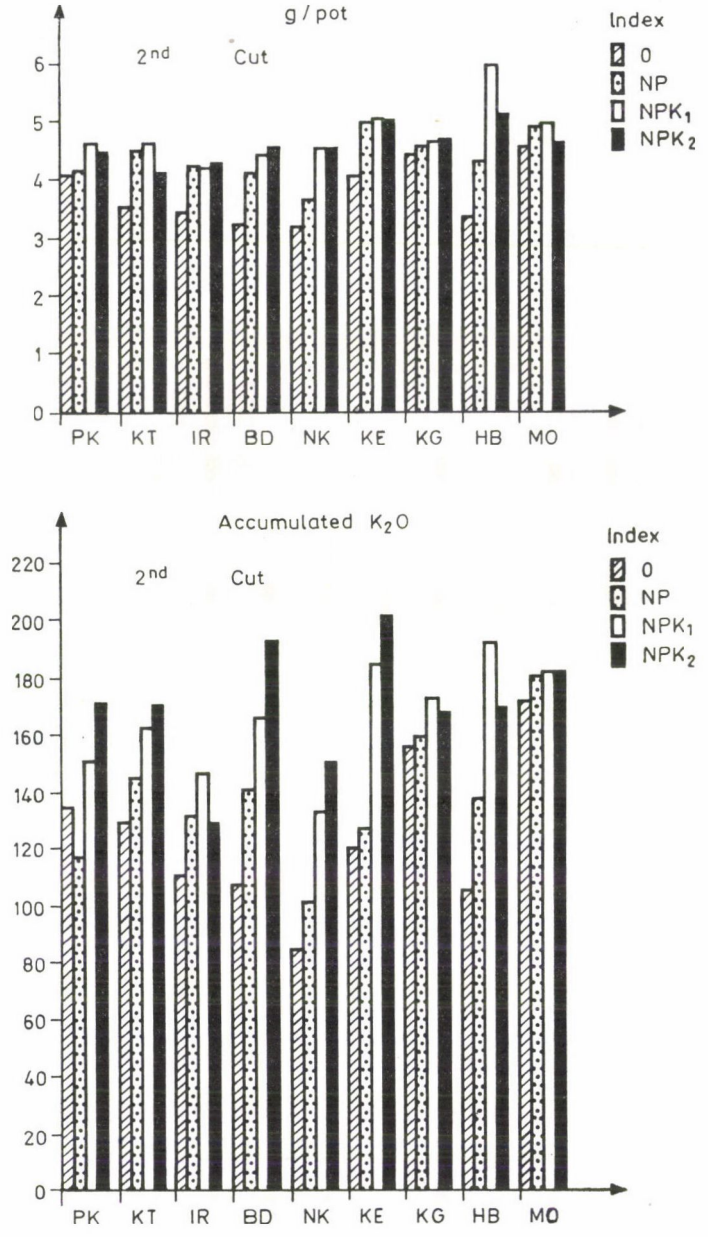


Fig. 2. Dry matter production of *Lolium perenne* and potassium uptake mg/pot

potassium uptake of ryegrass influenced by the big differences in K supply developed during the 16 years, showed considerable variations on different soil types.

In a part of the experimental soils there appeared no direct relationship between the level of potassium supply and dry matter production of plants. In the first cut, ryegrass plants produced the highest mass on the HB meadow,

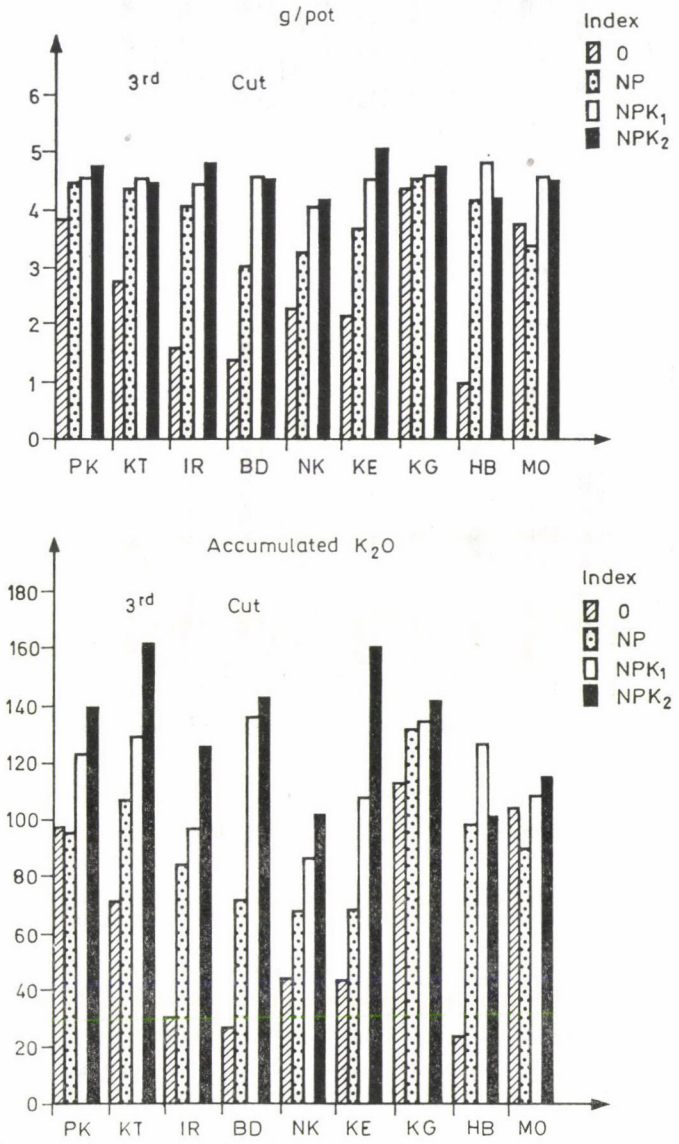


Fig. 3. Dry matter production of *Lolium perenne* and potassium uptake mg/pot

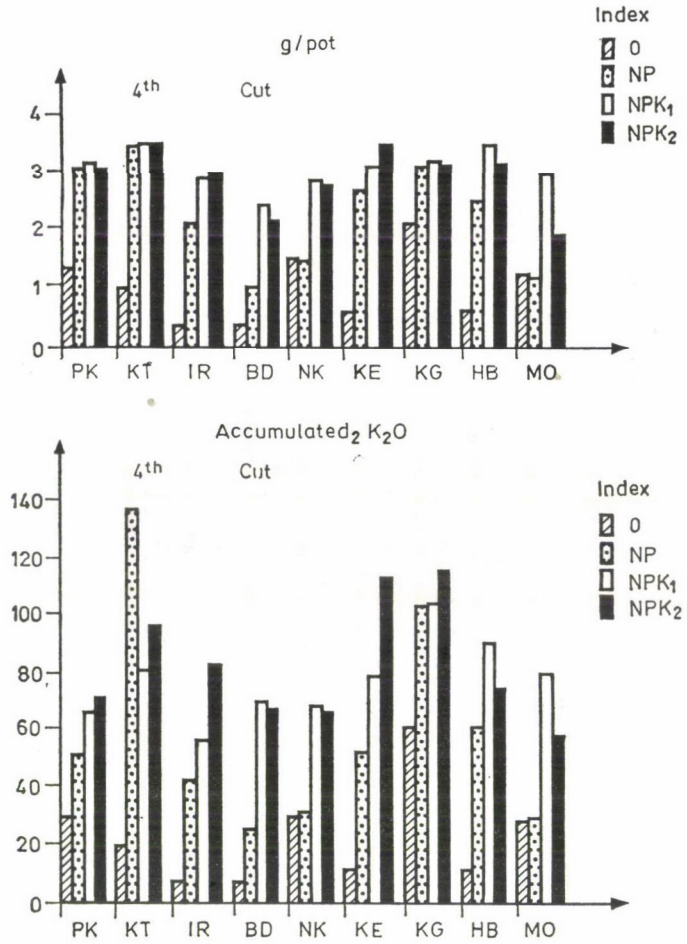


Fig. 4. Dry matter production of *Lolium perenne* and potassium uptake mg/pot

chernozem soil (4.31 g/pot). It was somewhat less in the second cut and a great reduction occurred in the third cut. Dry matter production reached its maximum — except for the meadow chernozem soil — in the second cut. It was followed by a gradual, then a sudden reduction in accordance with the level of supply.

On brown forest soils (PK, KT, KE), it can be observed already from the second cut that dry matter production is considerably higher than on certain chernozems (IR, BD). This especially appears from the third cut and probably reflects the better nutrient-supplying capacity of soils. Similarly, a prolonged nutrient supply was observed on MO carbonated fluvisoil in the control treatment. This can be seen on Figure 8, showing the total dry matter production of the 7 cuts.

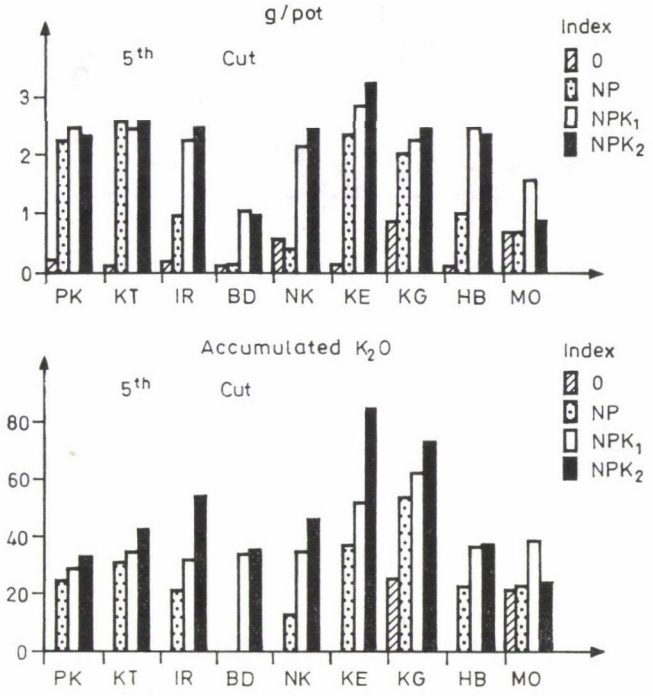


Fig. 5. Dry matter production of *Lolium perenne* and potassium uptake mg/pot

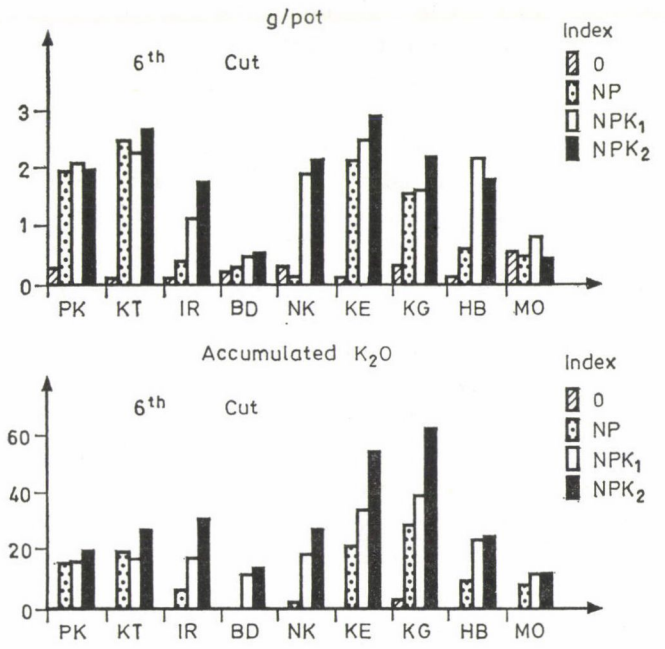


Fig. 6. Dry matter production of *Lolium perenne* and potassium uptake mg/pot

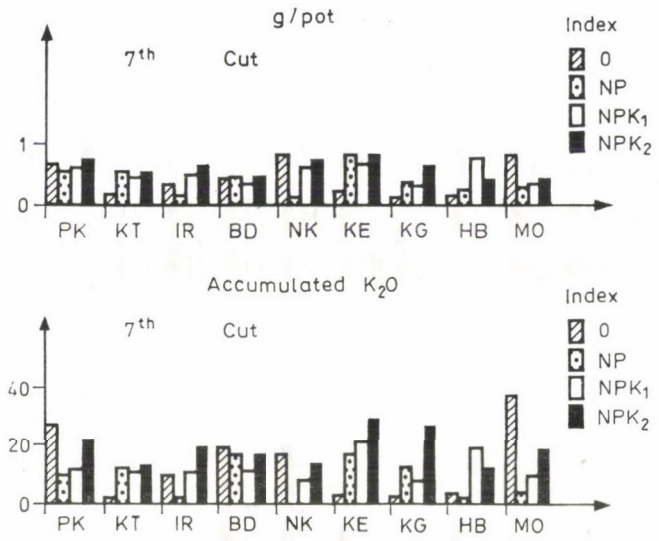


Fig. 7. Dry matter production of *Lolium perenne* and potassium uptake mg/pot

As the effect of NP treatment, dry matter production increased in most of the soils (e.g. KT chernozem brown forest soil) while it lessened in the case of others (e.g. HB meadow chernozem soil). It is interesting that the rate of decrease in KE brown forest soil was lower, so values determined at the sixth and seventh cuts were relatively high, as compared with the other soils. It is noteworthy that, in the NP treatment, the total dry matter production on 7 cuts were the highest in brown forest soils (KT, PK, KE) as is shown in Fig. 8.

The NPK_1 treatment resulted in a higher dry matter production on nearly every soil type, as the consequence of a more favourable nutrient supply. Dry matter production was the highest in this treatment at every cut on HB meadow chernozem soil, reaching its maximum at the second cut to be followed by a consistent decrease. Differences between the soils shown at the first cut were later relatively equalized, then developed again (see cuts 5—7.). The total dry matter production of the 7 cuts (Fig. 10) reflected approximately the same sequence of soils as found in the NP treatment. Ryegrass produced an outstandingly high dry matter yield (25.07 g/pot) on the meadow chernozem (HB) and brown forest soils (PK, KT, KE).

The NPK_2 treatment —a higher dose of potassium — did not produce a further increase in dry matter yield at the first two cuts (Figs. 1—6). However, the reduction of dry matter production from the third cut was more moderate.

It can be observed that the considerable difference between the soils was nearly equalized.

Taking the total dry matter production of the 7 cuts into consideration, it can be seen that the additional 100 kg/ha potassium could not be adequately utilized by plants. Bruchholz (1974) came to a similar conclusion based on

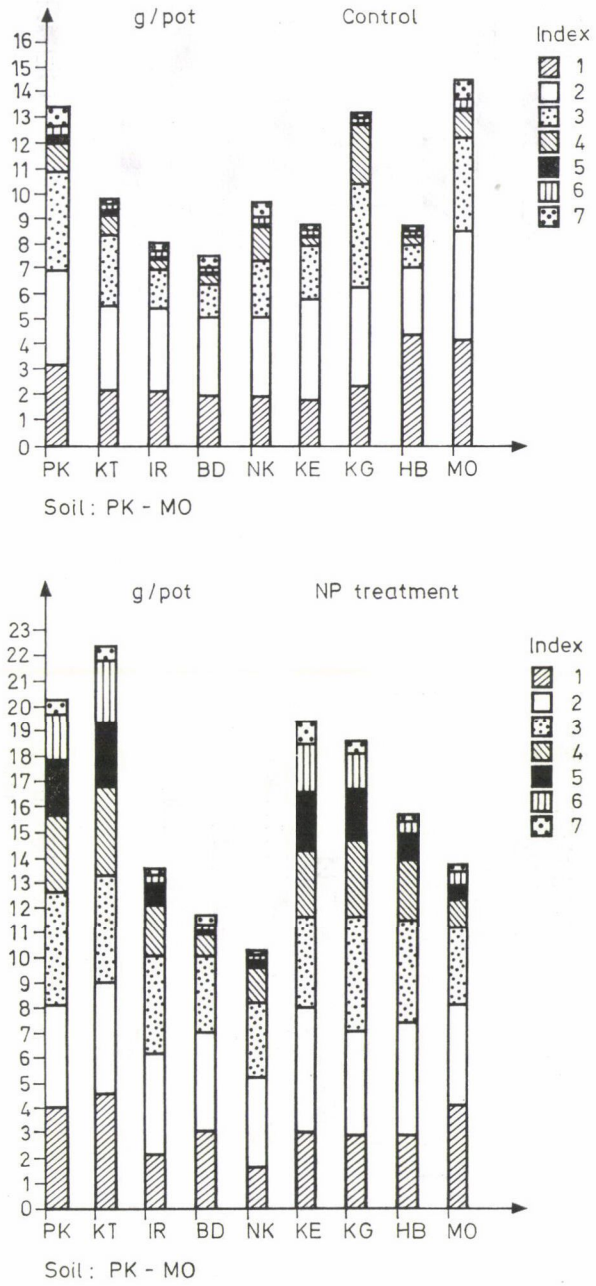
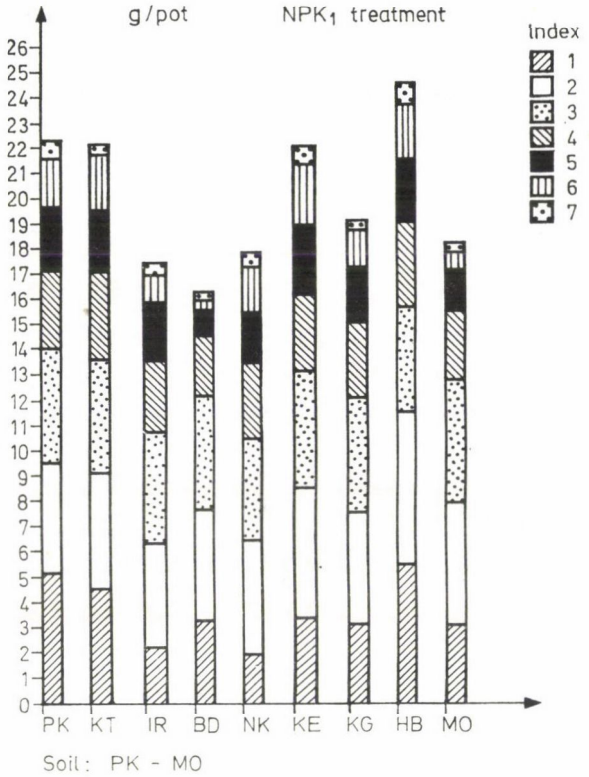


Fig. 8. Dry matter accumulation of *Lolium perenne*



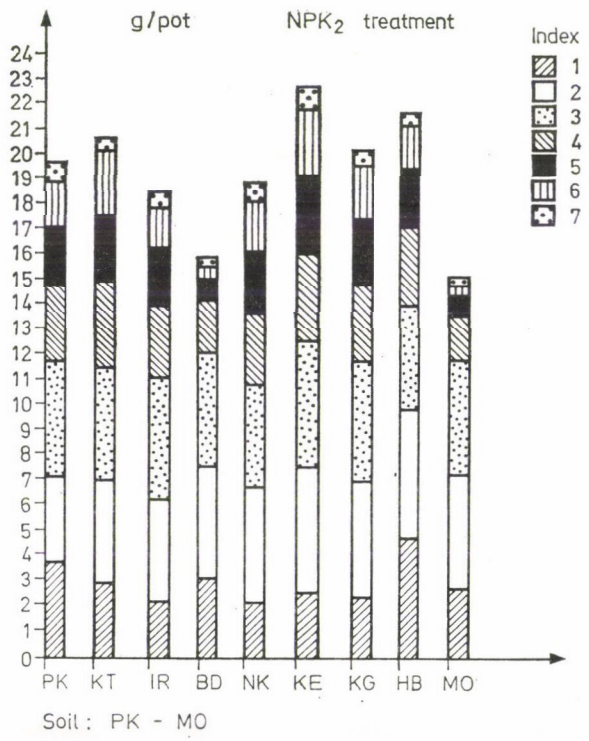


Fig. 9. **Dry matter accumulation of *Lolium perenne***

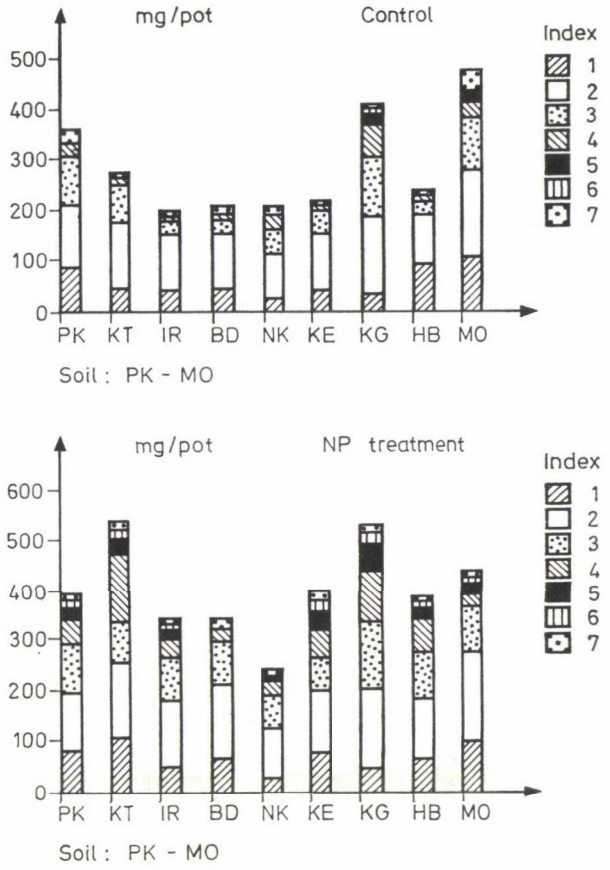


Fig. 10. Potassium accumulation of *Lolium perenne*

the results of a long-term potassium fertilization experiment in the GDR. The highest dry matter values, for the total of the 7 cuts, were measured on KE Ramann's brown forest soil (Fig. 11).

Differences observed in the potassium uptake of plants among soils and treatments were similar to dry matter production in most of the cases. These differences in the unfertilized control — according to the K supplying capacity of soils — formed an interesting sequence. At the first cut, the highest amounts of potassium were taken up by plants grown on MO carbonated fluvisoil and on HB meadow chernozem soil. However, from the second cut, potassium removal was the highest on the salt-affected meadow chernozem. This is by all means closely related to the higher clay mineral content of meadow soils and thus to their better potassium-supplying capacity. Taking the sequence into consideration by the amounts in potassium removal, the

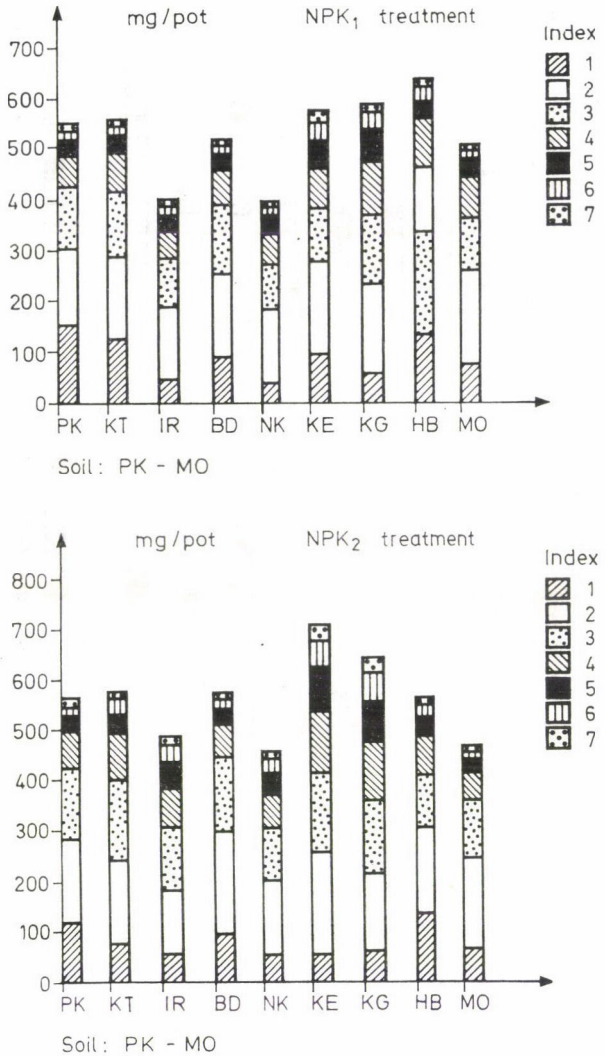


Fig. 11. Potassium accumulation of *Lolium perenne*

MO carbonated fluvisoil is the first, followed by the HB meadow chernozem and the PK chernozem brown forest soil.

As the consequence of NP treatment, potassium uptake markedly increased as compared with the control (e.g. on KT chernozem brown forest soil up to 105.5 mg/Kg_2O per pot) while it changed very little on others (e.g. on PK chernozem brown forest soil and on MO carbonated fluvisoil). The sequence taking shape from the second cut, remarkably reflects the ability of soils to supply K nutrient "in addition to" N and P for the balanced nutrient

supply of ryegrass. From the third cut, the effects of stable potassium supply on KG meadow chernozem soil was clearly detectable; potassium uptake was on an outstanding level (e.g. 29.73 mg K_2O pot at the sixth cut) on this soil, while it considerably decreased on others.

Brown forest soils also ensured a good potassium supply: summarized K_2O removal at NP treatment was the highest on KT chernozem brown forest soil (557.27 mg K_2O/pot).

As the result of NPK_1 treatments, potassium uptake considerable increased on most soils, which continued at the further cuts as well (e.g. KG meadow chernozem or KE Ramann's brown forest soil at fifth and sixth). Generally, it can be stated that a 100 kg/ha dose proved to be sufficient to plants and the 200 kg/ha dose (NPK_2 treatment) could not be utilized. This is evident if we compare K removal values measured in NPK_1 and NPK_2 treatments. As the result of the higher dose, potassium uptake somewhat increased on some soils but was not incidental to an increase in dry matter production referring to luxus consumption.

With the NPK_1 treatment, plants took up the highest amounts of potassium on HB meadow chernozem, while with the NPK_2 treatment, on KE Ramann's brown forest soil.

Discussion

Considering the dry matter production and potassium uptake of ryegrass, the following statements can be made:

- On chernozem soils (IR, NK) the dry matter production of ryegrass, in accordance with potassium uptake, rapidly increased, but it decreased markedly after the fourth cut.
- On brown forest soils (PK, KT, KE) dry matter production in the treatments with fertilizers stayed more balanced and reduction per cuts was moderate.
- The highest dry matter yield was produced on meadow chernozem (HB) in the NPK_1 treatment.
- The effect of higher potassium dose (NPK_2 treatment), compared with NPK_1 treatment, was negligible, which indicates that 100 kg/ha dose can already meet the potassium requirement of plants.
- The potassium mptake of plants — as one of the indicators showing nutrient supply — marked the available K content of the experimental soils and their K-supplying capacity. This is where the ability of soils to supply plants with nutrients from the non-exchangeable fraction can manifest itself. This resulted in good, marked differences in dry matter production and the potassium uptake of plants on several soil types in the treatment without K.

Similar results were obtained by Mengel and Wiechens (1979), who found that, following a decrease in exchangeable K level in the soil, ryegrass took nutrients mostly from the K ions in the interlayer positions of clay minerals, which was very important from the aspect of yield formation.

The results of the pot experiment can help in evaluating the potassium-supplying capacity of soils.

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MICROMORPHOLOGY AND SOIL FORMATION

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Soil micromorphology was used in the study of soil formation from its very beginning. The relations between the micromorphological characteristics and their forming processes contribute to a better understanding of soil formation. Some of these relations are discussed below and examples for their use interpreting the micromorphological investigations of alluvial meadow, meadow-, chernozem- and salt-affected soils are shown.

Keywords: Soil micromorphology, soil formation, alluvial-, meadow-, chernozem-, salt affected soils.

Introduction

Soil micromorphology is a branch of soil science having a special methodology: the microscopic investigation of undisturbed soil samples. Its history dates back to the publishing of Kubiena's book "Micropedology", exactly fifty years ago.

Soil micromorphology was applied by nearly all branches of soil science: soil-biology, -chemistry, -classification, -genetics, -geography, -mineralogy, -physics as well as cultivation etc.

From the very beginning an emphasis was laid on the use of micromorphology to elucidate soil forming processes. Different possibilities exist to reveal the relations between soil formation and soil micromorphological characteristics.

An example for one of the approaches will be given below, applying the revealed relationships between micromorphological characteristics and forming processes. Relationships between nearly all of the micromorphological features (e.g. microstructure, coarse and fine compounds including both mineral and organic ones, related distribution, pedofeatures) and forming processes were established (Bullock 1983, Brewer 1964, Brewer, Sleeman and Foster 1983, Romashkevich and Gerasimova 1982, Parfenova and Yarilova 1977 etc.). Due to the limited space of this paper only some of these relations are discussed here.

In general Brewer's terminology (1964) is used. The nomenclature from Handbook for Soil Thin Section Description (Bullock et al. 1985) is also given in brackets. In some cases, referring to the micromorphological literature, the term used by the author is given.

Method of micromorphological investigations

Examples for the interpretation of soil micromorphology to study soil formation, evaluating the investigations of meadow chernozem, alluvial meadow, meadow-, meadow solonetz, solodized meadow solonetz and solonchak soils, are inserted into the subchapters of the next title.

The data of these micromorphological investigations are given in Table 1. The headings follow the nomenclature of Handbook for Soil Thin Section Description (Bullock et al. 1985).

Beyond micromorphology the above mentioned soil profiles were studied by Darab et al. (1971), Jassó (1964), Szabolcs (1965), Szabolcs, Szendrei and Pártay (1980).

The soil thin sections were prepared as follows: the undisturbed soil samples were taken from each genetic horizons and dried at 40 °C in a drying oven. Then the samples were impregnated by a Hungarian-made polyester resin diluted with 50% aceton. A cyclohexan peroxide catalysator and a cobalt naphthanate accelerator were used. The diluting agent was evaporated in a fume cupboard. After sawing a plate of 4—6 mm thickness, one side was flattened and mounted onto glass slides. Later, the other side of the plate was thinned and finished with silicium carbide and carborundum grinding powders. Paraffine oil was used as a lubricant. Thin sections of 5—50 cm^2 size were prepared. The evaluation of thin sections was carried out using a polarizing microscope.

Results and discussion of micromorphological studies

The relations between micromorphological features and forming processes are discussed and the data of the micromorphological investigations of the studied soil types are interpreted under the heading of the selected micromorphological characteristics, as follows:

Plasma (fine material)

Plasma (Brewer, 1964) or fine material (Bullock et al. 1985) of soils were practically considered by micromorphologists as constituents below a grain size limit. Parfenova and Yarilova (1977) subdivided plasma according to its dominant constituents as follows:

— clayey,
— clay-organic,
— calcareous-clayey,
— ferruginous-clayey.

Plasma can be characterized by its extinction pattern as well. The superimposion of randomly oriented anisotropic fine particles (e.g. clay minerals) causes the compensation of their interference colours, whereas in the case of a parallel orientation of the particles in the domain it leads to reinforce the interference colours to a visible birefringence under crossed polarizers. These

domains should have different degrees of preferred orientation: sepic plasmic fabric — Brewer (1964) or b (birefringence) — fabric — Bullock et al. 1985 (Fig. 1). Only those which are mentioned in the text are included in the sketch.

The most evident reason of orientation resulting sepic plasmic fabric can be swelling and shrinking (due to wetting and drying). Greene-Kelly and Mackney (1970) carried out wetting and drying experiments with remoulded material (B_{tg} horizon of a fine textured soil). They did not recognize orientation

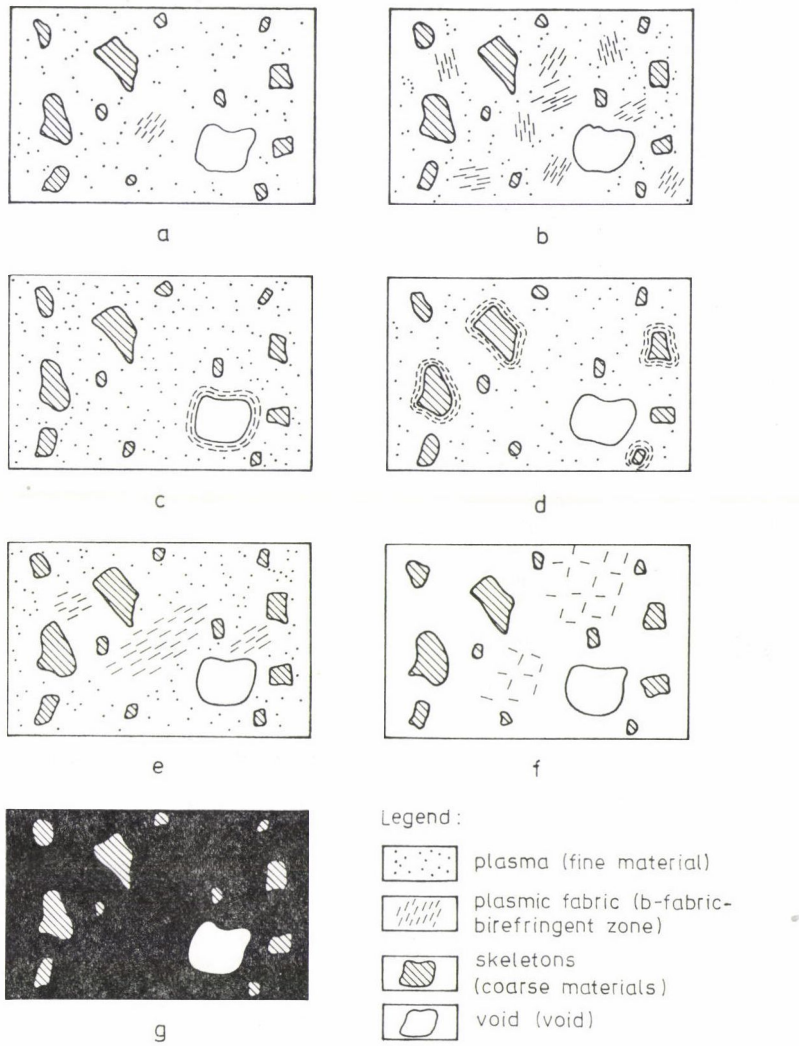

Fig. 1. The nomenclature of plasmic fabrics (birefrigence fabric) — idealized sketch: — Brewer (5962): a) insepic, b) mesepic, c) vosepic, d) skelsepic, e) masepic, f) lattisepic, g) isotic. — Bullock et al. (5985): a) latipple-speckled, b) stipple-mosaic-speckled, c) porostristed, d) granostristed, g) undifferentisted

Table 1

Micromorphological description of selected soil types

Soil type	Horizon	Microstructure: Aggregate type	Voids: Type			Voids: Size			Mineral and/or organic components: Coarse components				Fine materials: b-fabric				Related distribution of the fine and coarse material		Pedological features: Coatings, infillings		Nodule			Intercala
									Size										clay		Ferruginous		Calcitic	Gypsic
		Platy	Packing voids	Vughs	Planes	Macropores (100 μm >)	Mesopores (100-30 μm)	Micropores (30 μm >)	Mineral grains	1000–250 μm	250–50 m	50–10 μm	Undifferentiated	Crystallic	Stipple	Mosaic	Monic	Porpyric	Void compounds	Grain	Typic	Concentric	Typic	
Meadow chernozem	A				c	+	+		+		+	+	+					+	fw		fw		fw	
	B			f	c	+	+		+		+	+		+				+	fw		fw		fw	
	C			f		+	+		+		+	+		+				+		fw	fw		c	
Meadow soil	A			fw	fw	+	+		+		+	+				+		+			f			
	B_1			fw	fw	+			+		+	+				+		+	fw		c	fw		
	B_2			fw	f	+	+		+		+	+				+		+			c			
	C			f	fw	+	+		+		+	+				+		+			f		c	
Meadow alluvial soil	A			fw	f	+	+		+		+	+	+					+	fw	fw			fw	
	B			f	f	+	+		+		+	+		+				+	fw	f			fw	
	BC		f					+	+	+	+	+					+			f				
Meadow solonetz soil	A			f	f	+	+		+		+	+	+					+		fw				
	B_1			f	f	+	+		+		+	+				+		+	f	fw				
	B_2			f	f	+	+		+		+	+				+		+	f	fw				
	BC			fw	fw	+	+		+		+	+		+				+	f	f			fw	fw
	C		f	fw			+	+	+	+	+	+					+			f				

Meadow solonetz soil, solodized	A_1	+		c	c	+	+		+		+	+	+				+	fw		c		
	A_{1s}		c					+	+		+	+				+		fw		f		
	A_2			c	c		+		+		+	+			+		+	fw		c		
	A_{2s}		c					+	+		+	+				+		fw		c		
	B_1			c	c	+			+		+	+			+		+	fw		fw		
	B_2			c	fw			+		+	+				+		+	fw		fw		
	BC			f		+			+		+	+		+			+	fw		fw		fw
	C			fw		+			+		+	+		+			+	fw		fw		fw
Solonchak	1			fw	c	+	+		+		+			+			+	fw				
	2			fw	c	+	+		+		+			+			+	fw		fw		
	3			fw	fw	+	+		+		+			+			+	fw		fw		
	4		f			+	+	+	+	+	+					+				f		

Symbols: f = frequent, c = common, fw = few
A_s = solodized part of the horizon

due to wetting and drying but observed remarkable orientation by small stress even at low pF-values. Dalrymple and Jim (1984) have come to the conclusion that isotropic stress due to drying and wetting has given a rise to limited orientation (skel-, in- or partly mosepic plasmic fabric). Orientation of clay particles along the surface of skeleton grains was interpreted by Lafeber (1964) as due to rotation of particles.

The formation of lattisepic or masepic plasmic fabric in soil with higher expandability was deduced by McCormack and Wilding (1974), assuming shearing due to wetting and drying. The occurrence of developed plasmic fabric (ma-, vosepic) was elucidated by shearing due to pedoturbation (Blokhuis et al. 1970, Jongerius 1970). The crescent striated by-fabric (Bullock et al. 1985) was also interpreted by the activity of soil fauna (Fitz Patrick 1984). It is obvious that plasmic fabric should also be inherited from the parent material. Sepic plasmic fabric occurs in clayey rocks as well.

Concerning the main soil types of the Hungarian Lowland, surface horizons of alluvial-, meadow-, meadow solonetz and chernozem soils have organic-clay and the B horizons of meadow solonetz soils have clayey plasma. B_k or B_{Ca} horizons of alluvial-, meadow- and meadow solonetz soils possess calcareous clay or sometimes dominantly calcareous plasma. The development of the plasmic fabric of the soils under discussion is generally weak (a-, in-, sometimes mosepic), and indicates no high anisotropic stress.

Plasmic fabric depends on soil properties, primarily granular composition which was proved by the experiment of Dalrymple and Jim (1984) with artificial quartz sand-silt-Na/Ca bentonite mixture under isotropic stress, due to alternating wetting and drying. Increasing clay content promotes a more preferred orientation, whereas silt particles prevent it.

The enrichment of sodium ions in the adsorption complex increases the activity of clays. The limit value of clay content, indicating the appearance of developed plasmic fabric, was shifted to lower values.

Related Distribution of Fine and Coarse Constituents

The related distribution of coarse and fine materials is also one of the micromorphological characteristics indicating different aspects of soil genetics. Various concepts and terminologies of related distribution patterns were elaborated by Brewer (1964, 1976), Bullock et al. (1985), Eswaran and Banos (1976), Kubiena (1938), Stoops and Jongerius (1975). A simplified sketch is given in Fig. 2.

Searching for connections between related distribution patterns and soil properties, a relationship with granular composition seems to be feasible. Investigating more than a thousand soil thin sections of 95 soil profiles from Australia, Brewer (1979) established a relationship between the fabric and

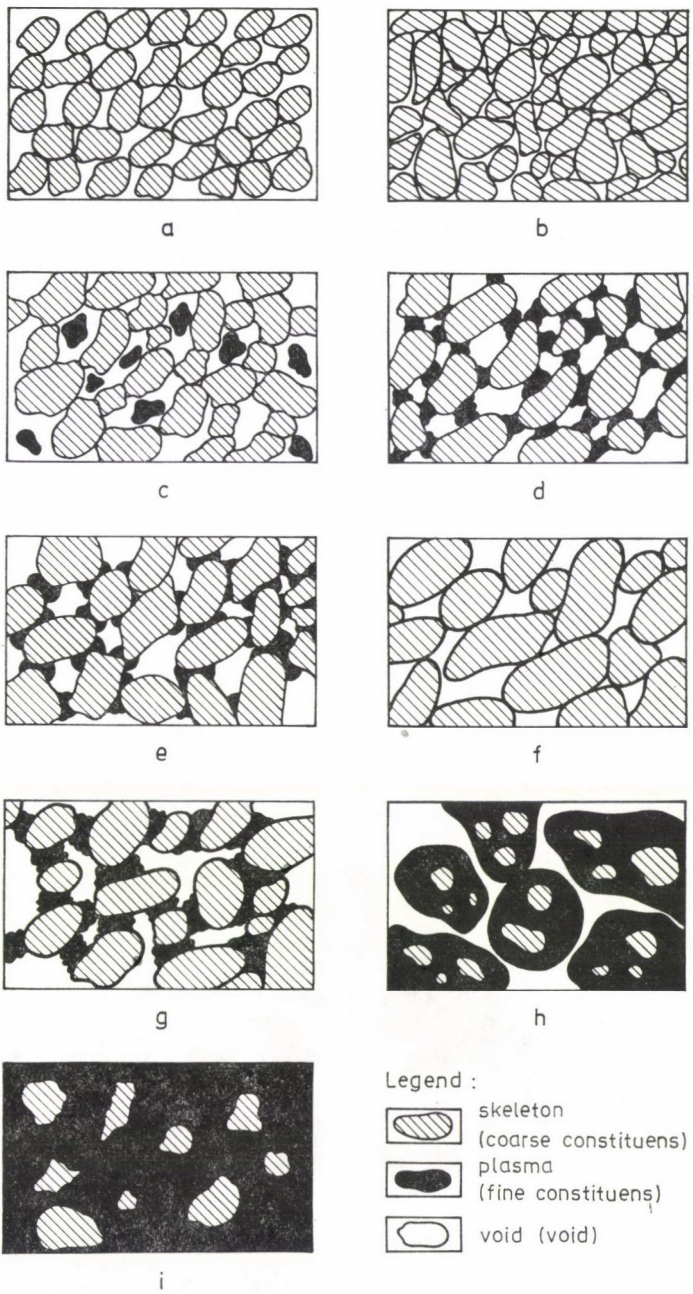

Fig. 2. The nomenclature of related distribution of coarse and fine constituens patterns- an idealized sketch: Brewer (5962): a, b) granular, c) agglomeroplasmic, d) intertextic, i) porphyroskelic, Brewer (1979): a) granic, b) fragmic, d) gefuric, e) iuctic, f) chlamydic, g) plectic, i) porphyroskelic, Eswaran and Banes (1976): a) granic, c) congelic, d) intertextic, f) dermatic, h) agglutinic, i) pophyric, Stoops and Jongerius (1975): and Bullock et al. (1985): a,b, monic, c) anaulic, d) gefuric, f) chitonic, i) porphyric.

particle size (in addition he referred to other factors such as the amount of carbonate microcrystals, of organic matter, or activity of soil fauna or illuviation processes). The fabric is porphyric above 23% silt plus clay. With decreasing silt plus clay, the fabric is plectic-porphyric or complex (silt plus clay = 20—23%), plectic (12—20%), chlamydic (< 12%)or orthogranic (< 2%), when silt/clay is below 1. When silt content is higher, the sequence is as follows: iunctic porphyric (15—23%), iunctic (< 15%) or orthogranic (< 2%). The former correlation was tested by McKeague and Guertin (1982) with some modifications for Canadian soils, based on the evaluation of 851 thin sections. Eswaran and Banos (1976) also recognized relation between texture and a normal related distribution pattern (a random distribution pattern). The *NRDP* (normal related distribution pattern) is granic when sand particles dominate over silt and plasma and is phyric when silt and is plasmic when clay dominates. When sand, silt and plasma occur in a balanced amount the *NRDP* is porphyric. Instead of random related distributed patters, a preferred, called specific distribution pattern *(SRDP)* can be formed due to pedogenesis. Plasma bridges (intertextic *SRDP*) between or coatings on skeletons (dermatic *SRDP*) can be observed, due to plasma translocation in sandy soils. Aggregates of sand, silt and plasma or of silt and plasma among the skeletons can be found in agglutinic or congelic *SRDP*, respectively.

Fig. 3 Granuar (monic) related distribution pattern of coarse and fine consatituens. Solonchak like meadow solonetz soil. C horizon. Magnification: 280 x, PPL:

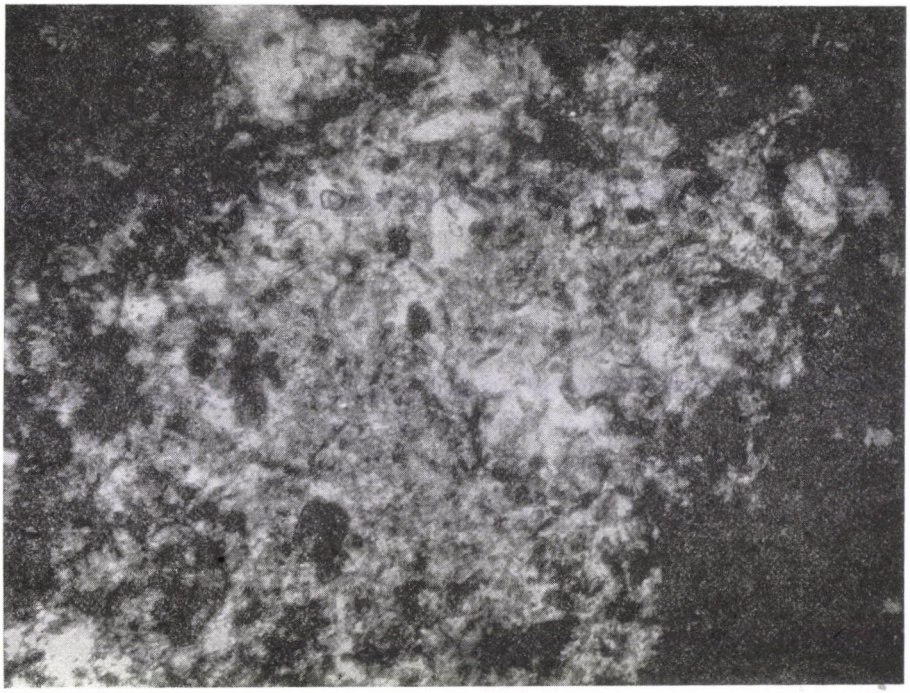

Fig 4 Granular (monic) and porphyroskelic (porphyric) c/f related distribution patterns. Solodized meadow solonetz soil, A—horizon. Magnification: 168x, PPL.

In the investigated soils of the Hungarian Lowland, i.e. in alluvial-' meadow-, chernozem- and salt-effected soils, the related distribution pattern is mainly porphyroskelic (porphyric). Sometimes it is granular (monic) indicating parent material heterogenity (*C* horizon of solonchak like meadow solonetz soil, Fig. 3) or reflecting an impoverishment in plasma (eluvial part of A horizon, solodized meadow solonetz soils, Fig. 4). In both cases the clay content was quite low.

Cutans (coatings)

Cutans can be differentiated according to their nature (argillans, sesquans, calcans etc.) as well. Of them some aspects of the formation of argillans (clay coatings) is detailed below.

Concerning their formation, different argillans, i.e. illuvial, stress oriented, neoformed, inherited from the parent materials and formed by weathering, were distinguished by Bullock and Thompson (1985). The most detailed study was devoted to the formation of illuvial argillans. Mobilization of clay particles requires an already dispersed but not yet coagulated system. Figures on the parameters (electrolite concentration, absorbed ions, pH, features of solid

phase etc.) can be obtained, e.g. from the papers of Oster, Shainberg and Wood (1980), and van Olphen (1963).

In addition the capacity factor is also decisive. Rebertus and Buol (1985) observed the micromorphological features of illuviation only when enough clay particles were available (in the mentioned case from the weathering of plagioclase in Dystrochrepts from USA). In fine loamy Hapludults of the studied soil sequence, plagioclase was already weathered, the supply of clay particles ceased, and therefore, illuviation stopped. The illuviation argillans appear again in clayey Hapludults, when biotite transforms into sand or silt-sized pseudomorphs, consisting of kaolinite; thus, clay particles are again available.

The clay can be accumulated by flocculation from suspension. In flocculated clay the platelets have no high degree of orientation (Eswaran and Sys 1979, Quirk 1978). Tessier (cit. van Ranstat al. 1980) carried out an experiment flocculating different clay minerals with $CaCl_2$ solution. Flocculated montmorillonite and illite had very weak orientation, observed by scanning electron microscopy, even after intensive drying. It was only kaolinite that expressed good orientation. The argillans separated from soil showed quite another image than flocculated clay minerals, i.e. fine, parallel compact layering with low porosity and smooth surfaces. The above mentioned consideration leads to the conclusion that the formation of argillans has another mechanism than flocculation.

The argillan formation is a physical deposition according to the terminology of Eswaran and Sys (1979). The following processes of physical deposition were distinguished:

(a) sieving,
(b) drying,
(c) gravitational deposition.

The deposition of clay particles by the above mentioned processes was proven experimentally (Dijkerman, Cline and Olson 1967, Brewer and Haldane 1957, Hallsworth 1963, Bond 1986 etc.).

The results of the above mentioned model experiments lead to some general conclusions; one of them is the fact that clay illuviation and formation of argillic horizons are closely related to grain size distribution.

Concerning the coagulating effect of Ca-ions, the formation of illuviation argillans was considered questionable in calcareous media. If this is accepted, the movement of clay particles can occur only after leaching of salts and calcium-carbonate. Moreover, Hallsworth (1963) and van Schuylenborgh (1972) considered the illuviation of clays saturated by Ca-ion, which is made possible in a medium of low electrolite concentration. Clay illuviation in calcareous soils was assumed with the leaching of clay particles by seasonal atmospheric waters from the overlying decalcified horizon until Ca-saturation

was reached (Aquilar et al. 1983). The fact that the already dispersed particles can move in calcareous horizons when the pores are large enough was indicated by Wieder and Yaalon (1978). The grain cutans and bridges were characteristic for clay illuviation in calcareous soils, namely in Israeli serozems, in cases when the structure was loose enough and the clay particles were not in close contact with microcalcite.

In the former cases, illuviation was described in calcareous soils but not in close contact with calcium-carbonates. In Spanish soils Aquilar et al. (1983) observed argillans on carbonate nodules, and calcite particles within illuviation argillans, and composed cutans consisting of argillans and calcitans.

In the investigated soil types of the Hungarian Lowland, clay coatings are rare in chernozem soils, indicating only an initial stage of negligible clay migration. Clay coatings are more frequent in meadow solonetz soils, particularly in their B horizons probably due to higher ESP. In meadow solonetz soils infillings with less orientation, and sometimes consisting of coarser particles, also occur. This fact is in accordance with the observation of Fedoroff and Courty (1986) in natric horizons. The clay coatings are again scarce in solonchak soils, due evidently to the higher electrolyte concentration causing flocculation.

Sesquioxidic nodules (ferruginous and manganiferous nodules).

The conditions influencing mobilization, migration and precipitation of ferruginous and manganiferous compounds (e.g. changing of wetting and drying, of reduction and oxidation, reaction (pH) of the medium, migration of mobile organic matter) are generally in connection with the water management of soils. Thus, the mechanism of ferruginous and manganiferous nodule (typic nodule) formation can be also discussed in this relation:

— unsaturated conditions:

— oxidation proceeds from ped surface to inside (drying after wetting).

According to Blume (1968) during reduction Fe-compounds are mobilized and migrate inwards to the aggregates. In the course of oxidation, O_2 diffuses among the aggregates causing precipitation as $Fe(OH)_3$.

In unsaturated soils with considerable water content, Veneman, Vepraskas and Bouma (1976) assumed the formation processes as follows. Reductive conditions are in the peds, where Fe-Mn compounds are mobilized and are migrating. Reaching the zone of channels and planes, where an oxidative condition predominates due to quick air movement, iron precipitates (see ferrans, hypo- and quasiferrans). The presence of ferran indicates partly reductive conditions in the voids as well, and wetter conditions than hypoferrans.

— Cases when drying is followed by wetting. The large voids (channels, planes etc.) are the places of quick water movement filling these voids, and producing reductive conditions and mobilization of iron and manganese on the surface, and their migration into the peds. Also due to drier conditions in the peds, the circumstances are more oxidative, causing a precipitation. Establishing saturation along the voids (channels and planes) there is a gleyed (hypoalban) followed by a quasiferran.

— Saturated conditions:

— In the course of a long saturation period micro morphological features of iron and manganese leaching (bleached colour) or loss of clay as well (albic neoskeletons) and low redox potential (Mn-nodules are absent or very rare) can be observed, as indicated by Veneman, Vepraskas and Bouma (1976), Vepraskas and Wilding (1983).

The different assumptions on nodule formation given above were supplied by field and laboratory experiments, as well (Veneman, Vepraskas and Bouma 1976, Vepraskas and Bouma 1976 etc.).

In situ nodules can be formed by cementing the matrix or by precipitating in voids. The latter are composed of relatively "pure" iron and manganese compounds free of impurities, having a shape often determined by the contours of voids. The former kind of nodules includes the particles of soil matrix.

Concerning the origin of the nodules, they can either be inherited from parent rocks (lithorelicts) or from other soils (pedorelicts).

In the latter case some transportation also has to be taken into consideration. When they are inherited, nodules should have sharp boundaries (Brewer, Sleeman and Foster 1983).

Different relations were recognized between the properties of typic nodules and the degree of water-logging in various soils, as follows (generalizations are valid only for some ranges, detailed discussion is not possible here):

— A positive correlation was found between the amount of nodules and the degree of wetness (Ogleznev 1968, Zaidelman, Gelcer and Nikiforova 1981, Zaidelman, Sanzharov and Polonskaya 1982, Evdokimova et al. 1984, Richardson and Hole 1979, Simonson and Boersma 1972).

— A shift of the highest amount towards the surface was observed in some cases (Schwertmann and Fanning 1976, Phillippe et al. 1972).

— An increase in size with the enchancement of hydromorphism was reported by Schwertmann and Fanning (1976), Simonson and Boersma (1972), Zaidel'man, Nikiforova and Sanzharov (1979).

— A stronger hydromorphic influence is reflected in a more diffuse boundary, as observed by Dobrovolski, Balabko and Kuzmenko (1981), Matinyan (1981), and Rudeforth (1970).

— A decreasing hardness with increasing wetness was reported by Tereshina and Nikiforova (1981).

— Relations were recognized between the chemical composition and forming processes as well. As mentioned before, the manganese oxide and oxihydroxide precipitate at higher redox potential (at more oxidative conditions) that of iron. Owing to this fact its presence indicates less reductive conditions and slighter wetting. Manganiferous pedological features (coatings and nodules) were observed by Veneman, Vepraskas and Bouma (1976) in horizons with short periods of water saturation; and horizons of long-term water-logging (for months) are characterized by the absence of manganese mottles in soils of Wisconsin toposequence. The same was observed in many other aspects of nodule formation. Sokolova and Polteva (1968) reported that iron compounds precipitate in podzolic A_1 and A_2 horizons and manganese ones in the lower part, due to the higher pH and Eh values.

The acid-soluble manganese content decreases and the amount of iron increases in the nodules with the enhancement of wetting (Ogleznev 1968, Zaidel'man, Sanzharov and Polonskaya 1982). The change in the Fe/Mn ratio is obviously reflected in the colour of the nodule.

Concerning the micromorphological investigations of the typical main soil types of the Hungarian Lowland, the ferruginous nodules are not characteristic for chernozem soils. In alluvial, meadow and salt-affected soils, due to hydromorphic influence, the grain coatings typic nodules are common or

Table 2

Micromorphological description of ferruginous and manganiferrous nodules

Soil type	Horizon	Colour	Boundary	Size		Degre of hydromorhic influence	Abudance
				100 μm >	100 μm <		
Meadow soil	A	yb	d	+		3	f
		yb	d	+		3	c
	B_1	yb	sh	+		2	fw
		b	sh	+		1	fw
	B_2	yb	sh	+		2	c
	C	yb	d	+		3	f
		b	sh	+		1	fw
Solodized meadow solonetz soil	A_1	bb	cl	+		2	f
	A_2	bb	cl	+		2	c
	B_1	yb	sh	+		2	fw
	B_2	yb	d		+	3	fw
	BC	b	sh	+		1	c
	C	b	sh	+		1	c

Symbols: yb = yellowish brown, bb = brownish black, b = black, d = diffuse, cl = clear, sh = sharp, f = frequent, c = common, fw = few.

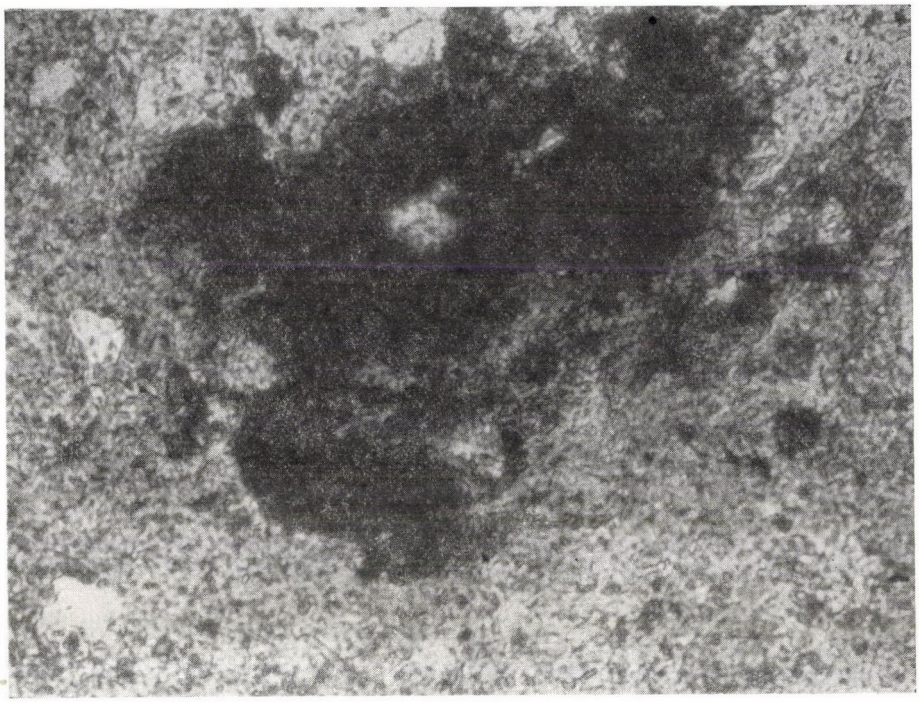

Fig. 5 Nodule (typic nodule) Meadow solonetz soil, B_1-horizon. Magnification: 208x, PPL.

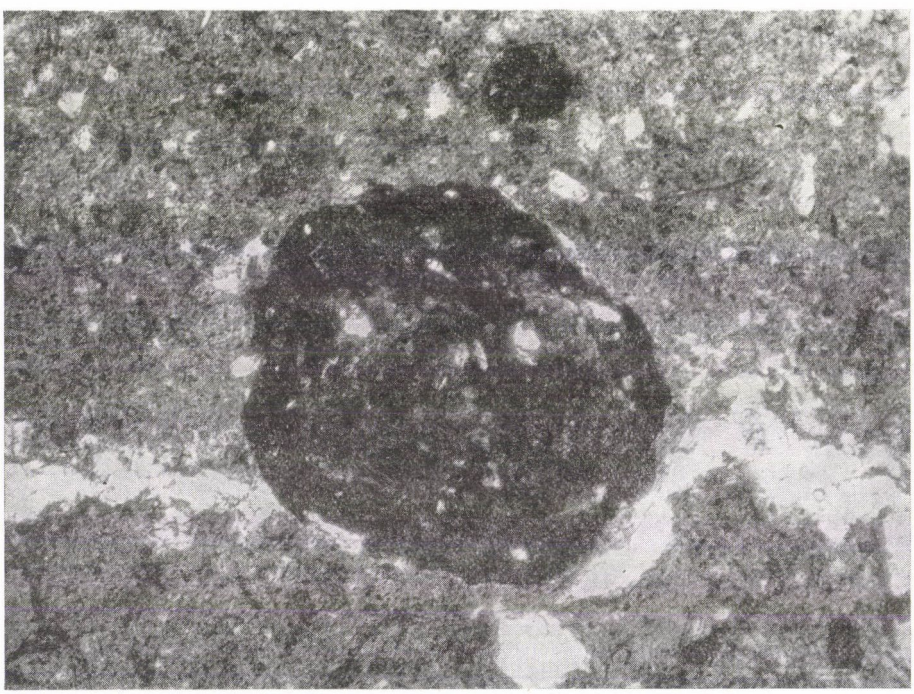

Fig. 6. Concretion (concentric nodule). Medow solonetz soil, B_1-horizon. Magnification: 208x, PPL.

frequent (Table 1). Some micromorphological properties of the nodule (colour, boundary, size) are shown in Table 2 to make possible the interpretation of this micromorphological description from the aspects of degree of hydromorphism. Micromorphological characteristics reflecting the increase of wetness are indicated with the increasing number of points. The points are summarized and grouped to 1, 2, 3 with the increasing degree of hydromorphism. Sometimes, the evaluation indicates different "generations" of nodules in the same horizons, referring to processes with varying degrees of hydromorphism. All of the investigated meadow and salt-affected soils have horizons with nodules indicating strong hydromorphic influences (Fig. 5).

The formation of concretions (concentric nodules) is assumed in a medium with alternating wetting and drying (Brewer 1964, Brewer, Sleeman and Foster 1983, Parfenova and Yarilova 1977 etc.). Concretions were observed in meadow soil of the Hungarian Lowland (Table 1, Fig. 6).

As it was shown before applying the relationship between forming processes and micromorphological features, the latter can be interpreted from the aspects of soil formation to elucidate the processes involved.

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PHOSPHATE SORPTION ON NARRABI SOIL EVALUATED BY LANGMUIR ADSORPTION AND SOLUBILITY ISOTHERMS

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Phosphate sorption on Narrabi soil was shown to be described by a four-region *Langmuir* equation, i.e. the plot showed four distinct linear portions. The equilibrium P concentration against the break in the slope of the *Langmuir* plots were found on a Solubility diagram of calcium-phosphates, to corresponded closely to saturation with respect to hydroxyapatite, β-tricalcium phosphate, octocalcium phosphate and dicalcium phosphate dihydrate respectively. Thus deviation occurred in the *Langmuir* plots were due to formation of these different calcium phosphate compounds. The adsorption maxima enhanced with P concentration and with increasing equilibration period used for sorption study. The bonding energy term computed from *Langmuir* plots decrease with amount of P sorbed, but gave no consistent trend with equilibration period. Partial molar free energy of phosphate anion ($\Delta\bar{G}_{Ca(H_2PO_4)_2}$ Cal./equiv) indicate that at an equivalent amount of P sorbed, the bonding energy for P held by soil enhanced in P sorption but declined in P desorption process with increased equilibration period.

Keywords: *Langmuir* isotherm, solubility isotherm, partial molar free energy.

Introduction

Phosphate sorption isotherm describes the relationship between the amount of P sorbed by the soil and the equilibrium solution P concentration at a given temperature. Often, the *Langmuir* equation has been used to characterize the adsorption of P by soils (Olsen and Watanabe 1957; Shapiro and Fried 1959 and Gunary 1970) and soil minerals (Cole et al. 1953; Hingston et al. 1957). A major advantage of the *Langmuir* equation is that it is possible to compute an adsorption maxima and a relative binding energy term for P sorption. Deviations from a single linear *Langmuir* plot in soils, soil minerals and resin surfaces have been reported due to P adsorbed with different energies of binding on the surface of adsorbent (Griffin and Jurinak 1973 and Syers et al. 1973) or due to different points of attachment by the phosphate anion (Taylor and Ellis 1978). Most of these studies, confined to homogeneous mineral or resin surfaces and with a limited range of P concentration to sil are indicated either by a straight line or with a single break in the *Langmuir* plot. These workers used shorter as well as different reaction periods, while completely

ignoring the influence of equilibration period on sorption process and eventually on their *Langmuir* parameters.

The purpose of this research was to investigate the sorption of phosphate on Narrabi soil, determined after different equilibration periods by combining *Langmuir* isotherm data with solubility equilibria.

Materials and methods

The soil used was a clay loam (64% clay) with pH (H_2O) 8.1, cation exchange capacity = 468 m-equiv/kg and EC = 0.141 mS/cm for a soil water extract. For P sorption, 5 g soil in duplicate was equilibrated for 2, 7, 13 and 64 days in 200 ml plastic bottles containing 100 ml of 0.01 M $CaCl_2$ solution, with varying P concentrations up to 65 μg P/cm^3. Sorption isotherms were determined by equilibrating soil solution suspensions in a room maintained at 22 °C. After the equilibration period, a clear solution was separated from the suspension by means of a millipore microfibre-glass filter (0.22 μm) paper assembly. The pH and EC were measured in the filtrate solution at 25 °C by pH meter and conductivity bridge, respectively. Phosphate was determined by the method of Fogg and Wilkinson (1958) and the P sorbed was computed from the difference between initial and final concentrations. Filtrates were also analyzed for calcium by varian atomic absorption spectrophotometer 975.

The *Langmuir* equation (1918) was used to interpret the equilibrium sorption data. The linear form of *Langmuir* equation is:

$$C/X = \frac{1}{kb} + \frac{C}{b} \tag{1}$$

where C is the equilibrium P concentration (μg P/cm^3); X, is the amount of P sorbed (μg P/g soil); b is the sorption maxima (μg P/g soil) and k is constant related to the energy of binding for P sorption (cm^3/μg P).

For solubility isotherms, the lime potential (pH − 0.5 pCa) and monocalcium phosphate potential (pH_2PO_4 + 0.5 pCa) were determined for soil equilibrated with a solution containing a varying amount of P. Ion activities were calculated using an iterative program employing the Davies equation (Davies 1962) and EC was used as an estimate of ionic strength. Corrections for the following ion pairs were made: $CaHPO_4^0$, $CaH_2PO_4^-$, $CaCO_3^0$ and $CaHCO_3^-$. All equilibrium constants used for ion pairs and mineral isotherms were selected from Lindsay (1979). Phosphate minerals considered were hydroxyapatite $Ca_5OH(PO_4)_3$, β-tricalcium phosphate β-$Ca_3(PO_4)_2$, octocalcium phosphate $Ca_8\ H_2(PO_4)_6 \cdot 5H_2O$, and dicalcium phosphate dihydrate $Ca\ HPO_4 \cdot 2H_2O$.

The bonding energy in terms of partial molar free energy of phosphate anions ($\bar{\Delta G}_{Ca(H_2PO_4)_2}$ calories/equiv) in equilibrated solutions determined after 2 and 64 days and for the different break sites was computed by the following equation:

$$\bar{\Delta G}_{Ca(H_2PO_4)_2} = -2.303RT\ (0.5pCa + pH_2PO_4)$$

where R is the molar gas constant (1.987 KCal/mole), T is absolute temperature (295 °K) and (0.5pCa + pH_2PO_4) is the monocalcium phosphate potential.

Results and discussion

Langmuir isotherms

Langmuir plots of phosphate sorption on Narrabi soil, determined after different equilibration periods, are shown in Fig. 1. Three different breaks in the slope of each *Langmuir* plot showed four distinct linear portions. The P

sorption maxima increased with P concentration and with reaction period for all the four regions (Table 1). The bonding energy for P sorption was maximum in region I, and it declined in the regions II, III and IV, for all the isotherm plots determined after various reaction periods.

The different slopes in Fig. 1 may be due either to adsorption taking place at energetically different sites on the surface, or to precipitation of the phosphate anion in the form of different calcium phosphate compounds.

Table 1

Langmuir parameters for P adsorption on Narrabi medium clay loam soil determined after different equilibration periods

Equilibration period (Days)	Regions							
	I		II		III		IV	
	k	b	k	b	k	b	k	b
2	0.574	202	0.363	263	0.125	447	0.086	537
7	0.749	215	0.219	346	0.131	546	0.057	805
13	0.825	231	0.350	370	0.148	575	0.061	841
64	0.651	304	0.314	437	0.140	643	0 067	906

k = bonding energy $cm^3/\mu g$ P; b = adsorption maxima $\mu g/g$ soil.

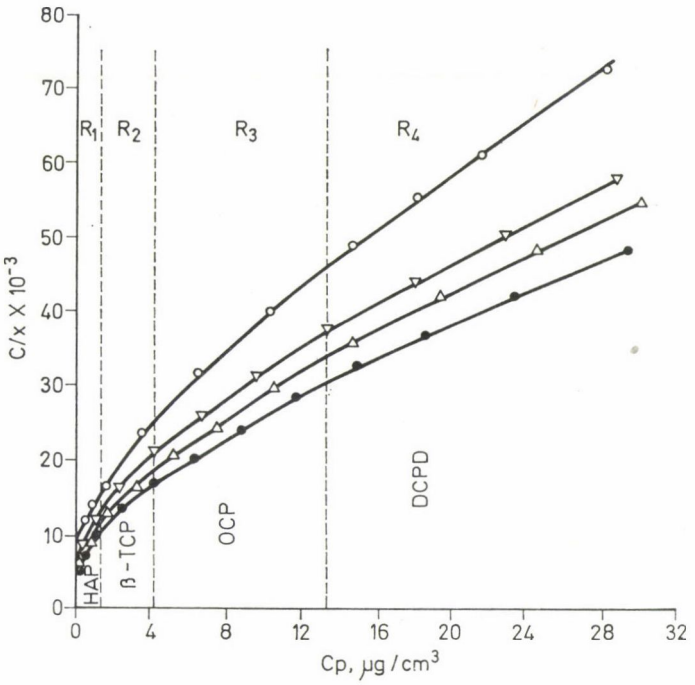

Fig. 1. Langmuir isotherm for P adsorption on Narrabi medium clay loam soil determined after 2, —o—o, 7, —×—×— 13, —△—△—; and 64, —●—●—; days

At low P concentration, P adsorbed with different energies of binding on homogenous soil mineral surfaces (Mljadi et al. 1966 and Griffin and Jurinak 1973). The relatively low P concentration at which deviations in these studies occurred should exclude precipitation, but there is always the possibility that native P minerals might induce precipitation during P sorption.

Solubility isotherm

Whereas true thermodynamic equilibrium conditions do not exist and solubility criteria are often not strictly applicable, it is nevertheless interesting to apply these criteria to the P sorption data. Lime potential (pH — 0.5 pCa) and phosphate potential, determined for the three break points of the sorption isotherm after different reaction periods (Table 2), are plotted on a solubility

Table 2

The value of (pH — 0.5 pCa) and (pH_2PO_4 + 0.5 pCa) for Narrabi medium clay loam soil observed at three break sites of Langmuir isotherms

	Final equilibrium P concentration $\mu g/cm^3$	(pH-0.5pCa)	(pH_2PO_4 + 5pCa)	$\Delta\overline{G}_{Ca(H_2PO_4)}$ (Cal/equiv)
No P	0.070	6.29	7.810	—10650
1st break	1.40	6.26	6.751	—9206
2nd break	4.20	6.16	5.890	—8031
3rd break	13.20	5.91	5.250	—7159

diagram (Fig. 2). From this diagram, the break points in the slope of the *Langmuir* plots corresponded roughly to the different calcium phosphate that may be obtained as reaction products. These are regulated by the chemical potential gradients of the phosphate anion established by the relative supersaturation of the initial solution. For region I, at a very low P concentration, the soil is saturated with hydroxyapatite, and it is likely that precipitation occurs as this compound. In region II, III and IV of the *Langmuir* plot, the soil was saturated with β-tricalcium phosphate, octocalcium phosphate and dicalcium phosphate dihydrate, respectively. Therefore, it appears likely that deviations from linearity for the Langmuir plots of P sorption, determined after various reaction periods on Narrabi soil, were due to formation of separate phases of calcium phosphates.

Relationship between partial molar free energy of phosphate anion ($\Delta\overline{G}_{Ca(H_2PO_4)_2}$ *Cal./equiv)* and *P sorbed*

The bonding energy for P sorption computed from the *Langmuir* plots decreased with the higher amount of P sorbed, but gave no consistent relation-

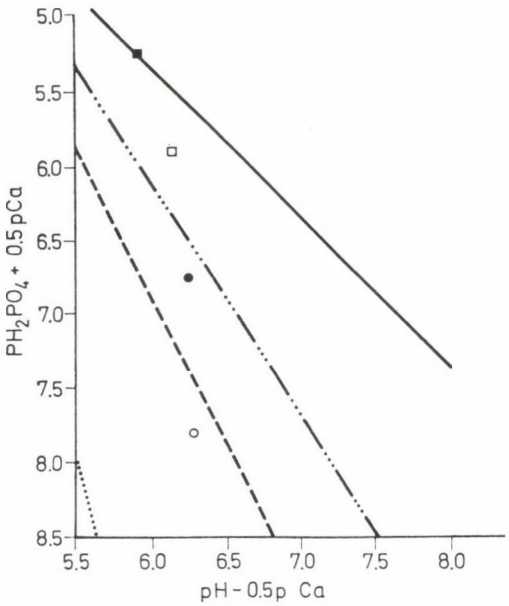

Fig. 2. Solubility diagram of the calcium phosphates (·····, hydroxyapatite; — — — —, β-tricalcium phosphate, —···—···—, Octocalcium phosphate; ————; dicalcium phosphate dihydrate) with equilibrium P concentration observed for ○, NOP; ●, first break; □, 2nd break; ■, 3rd break sites observed on the Langmuir plot for P adsorption isotherms

ship with the equilibration period. The bonding energy term should have increase with the equilibration period, but declined with the amount of P sorbed and thus a reversal effects of these two factors produced no consistent trend with the equilibration period. Therefore, the effect of equilibration period was studied in terms of relationship between partial molar free energy of phosphate anion ($\overline{\Delta G}_{Ca(H_2PO_4)_2}$ Cal./equiv) and P sorbed (Fig. 3). At an equivalent amount of P sorbed (> 12.5 μg g^{-1} soil), the bonding energy for P sorption was more after 64 than after 2 days. The bonding energies determined at different break sites (Table 2) also indicate an inverse relationship with equilibrium P concentration. The slope of the curve of $\overline{\Delta G}_{Ca(H_2PO_4)_2}$ versus P sorption differs on either side of point at 12.5 μg P/g soil. This indicates that a process of desorption differs from that of sorption, and the bonding energy of P held by soil decreases with the equilibration period, where a desorption process is taking place. Thus the equilibration period reduced the bonding energy of P held by soil during the dissolution process, but enhanced the bonding energy of P sorbed during the P sorption process.

Conclusions

The deviations occurred in the *Langmuir* plot for P sorption, determined after various equilibration periods, were due to the formation of different

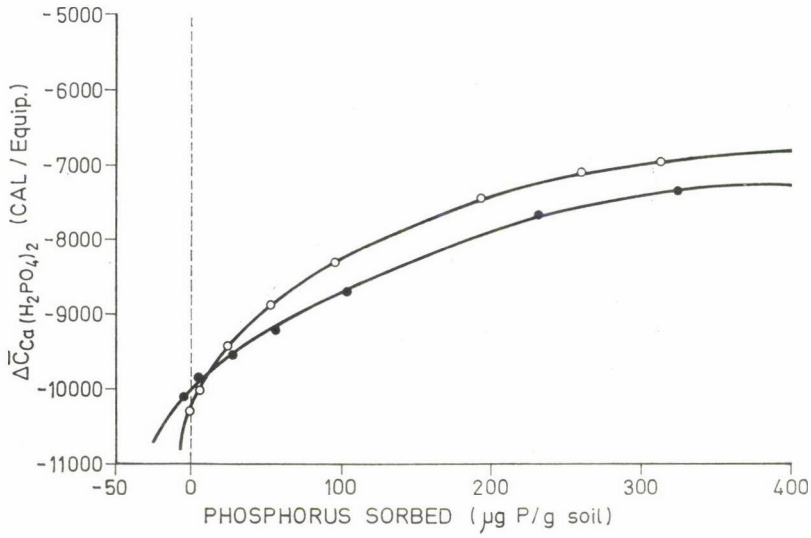

Fig. 3. Partial molar free energy of phosphate anion ($\Delta\bar{G}_{Ca(H_2PO_4)_2}$ Cal./equiv) and phosphorus absorbed determined after 2, o—o; and 64, •—•; days

calcium phosphate compounds. The P sorption maxima increased with the initial P concentration and with the equilibration period. The bonding energy term determined by Langmuir equation indicates an inverse relationship with the P concentration, but gave no trend with equilibration periods. $\Delta\bar{G}_{Ca(H_2PO_4)_2}$ indicates that bonding energy for P held by soil declined in desorption, but increased in sorption processes with increasing reaction periods.

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Plant physiology and biochemistry

THE RELATIONSHIP BETWEEN WATER SUPPLY, FOLIAGE TEMPERATURE AND YIELD IN SNAP BEANS

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The effect of different amounts of water supply on snap bean yield was investigatedin experiments over several years. Our research work was assisted by an infrared remote thermometer for measuring foliage temperature. Besides our yield parameters these data also indicate that irrigation carried out at different times improves the water balance of plants in different ways. Transpiration maintained at an optimal level ensures optimal foliage temperature even in the case of high air temperatures.

The effect of different water supplies is well manifested in the daily foliage temperature and also in the dynamics of vegetation period. There might be an 8 to 10 °C difference in the foliage temperature of snap beans that receive either a minimal or an optimal supply of water. The effect of these water supplies is especially notable between noon and four o'clock in the afternoon. In the case of an optimal water supply, the foliage temperature is sometimes much lower than the air temperature. If the beans suffer from a lack of water, the foliage temperature might be higher by 2 to 4 °C than the air temperature.

The different characteristics of foliage temperature measured daily are in close correlation with the forming plant weight (biomass) and also with the yield. The closest is the negative linear correlation with the sum of foliage temperatures above 20 °C ($r = -.9364$ and $r = -.8751$), with the maximum of foliage temperature ($r = -.9325$ and $r = -.9328$), with the number of days having a temperature above 30 °C ($r = -.9508$ but with the yield only $r = -.7722$). The differences between foliage and air temperatures represent also a close correlation with the quantity of plants and their yield.

Keywords: snap bean (*Phaseoulus vulgaris* L.), irrigation, foliage temperature' yield and plant quantity.

Introduction

Irrigation investigations were carried out in order to determine the correlation between water requirements and environmental conditions. We investigated the effect of different water supplies and of irrigation on yield formations. We paid particular attention to the correlations between temperature, water transport and supply from the beginning of our research.

The close correlations between water transport and air temperature in the case of snap beans was pointed out by Cselőtei (1959) using well advanced plant stands. Varga (1977) found a close correlation between the product of

accumulated temperatures, daily average temperatures and water use. The change of water transport affected by temperature during the growing period was determined in experiments upon plants that were sown at different times (Varga 1974, 1977, Cselőtei and Varga 1987).

The different characteristics of irrigation of snap beans sown during the spring and summer are also in correlation with the differing temper.ature and precipitation of the vegetative period (Varga and Obreczán 1988) The effect of water supply and temperature is manifested not only in yield quantity but quality as well (Cselőtei and Varga 1988).

The application of infrared remote thermometers was initiated by Tanner (1963). If soil humidity is suitable for the plant than the value of plant and air temperature difference is near zero, or negative (Idso et al. 1981). Bonano and Mack (1983) used air and plant temperature differences to predict snap bean irrigation and to estimate yield. Cselőtei and Helyes (1988) established that foliage temperature data measured in the early afternoon (13:00 to 15:00) are the most adequate for the characterization of water supply. If the plants are unable to draw sufficient water from the soil, necessary for their transpiration, the foliage temperature will then be higher than the air temperature. This positive temperature difference increases according to the lack of water and the low level of soil moisture (Helyes 1989).

Materials and methods

The formulation of snap bean stand temperature with different water supplies was investigated in three factorial experiments repeated four times at the Training and Experimental Farm of the Department of Horticulture of the University of Agricultural Sciences.

Weather conditions of the experiments are represented by the data of Table 1.

Table 1

Characteristics of weather during the vegetation periods of experimental years

Month	1986		1987		1988	
	Average temp. °C	Precipitation mm	Average temp. °C	Precipitation mm	Average temp. °C	Precipitation mm
V.	18.7	11.9	13.6	121.0	16.0	75.0
VI.	19.6	45.4	18.8	55.3	18.1	61.5
VII.	20.8	48.5	21.9	42.1	21.9	16.2
VIII.	21.8	31.0	17.4	103.3	20.1	119.6
IX.	17.0	0.9	18.2	30.2	15.8	74.1

The effect of different water supplies on plants are represented by the results of experiments with spring and summer sowing. Table 2 contains the characteristics of variety Valja and the dates of irrigation. Irrigation was done by sprinkler systems, with a dosage of 40 mm each time.

The experimental site had a brown forest soil, with a mechanical composition of adobe and sand. Its ability to retain water was (FC%) 22—23 vol.% in the ploughed layer and 13—15 vol.% in deeper layers. The value of useful water content decreases with depth from 15—16 vol.% to 8—10 vol.%.

The raynger II. infrared remote thermometer is a hand thermometer operating with a 9 V battery that is able to measure the temperature of objects without touching them, a significant development compared to earlier ways of measuring plant temperatures. Temperature measurement is independent from distance up to a certain point; if the measured object is larger than the lens diameter, one can measure a surface of 3 cm in diameter from 1 m and 30 cm from 10 m. The device collects 99% of infrared energy emitted by the object, which provides a temperature measurement with ±1% exactness. The measuring velocity

Table 2

The characteristics of plant growth during irrigation experiments with snap bean
Variety: Valja

Phenological phases	Spring sowing			Summer sowing	
	1986	1987	1988	1986	1987
Sowing	5.16.	5.18.	5.18.	7.16.	1.14.
Germination	5.23.	5.30.	5.27.	7.23.	7.20.
Floweing	6.27.	7.02.	6.29.	8.22.	9.01.
Picking	7.29.	7.24.	7.22.	9.29.	9.24.
Dates of irrigation					
1. Unirrigated	—	—	—	—	—
2. Irrigated before flowering	6.20.	7.01.	6.27.	8.04.	8.31.
3. Irrigated after flowering	7.02.	7.08.	7.05.	8.27.	9.09.
	7.11.	7.17.	7.13.	9.10.	9.18.
4. Regularly irrigated	6.20.	7.02.	6.27.	8.04.	9.01.
	7.04.	7.14.	7.06.	8.19.	9.09.
	7.15.	7.22.	7.13.	9.04.	9.17.
				9.13.	

of the thermometer is rather high, registering 4 data within a second and displays them digitally. The emission level should be set before use. Tanner (1963) found the emission of green plants between 0.95—0.97 and the emission of bean was 0.967. Our measurements were carried out at 97% emission level.

The temperature of plant stands with different water supplies can be compared only if measurements are done at the same air temperature and when radiation conditions are also the same (Helyes and Varró 1987). Accordingly, parallel to the measurements of plant temperature, we also determined global radiation with the Janisevszky radiation meter. Air temperature was determined by a digital thermometer of Keithley 886 in the height of the plant stand.

We determined these measurements at 13:00 every day during the vegetation period. The experimental designs used were randomized complete block (RCB), each with 4 replications. All plots were 80 m^2. The periodically harvested territory of each analysed variety was 2 m^2, which involved 80—90 plants for every replication.

Results and discussion

The vegetation period and mainly the precipitation during the yield formation period significantly affect the snap bean yield in Hungary. Natural water supply in most years is insufficient for foliage, plant weight and thus also for the necessary yield formation.

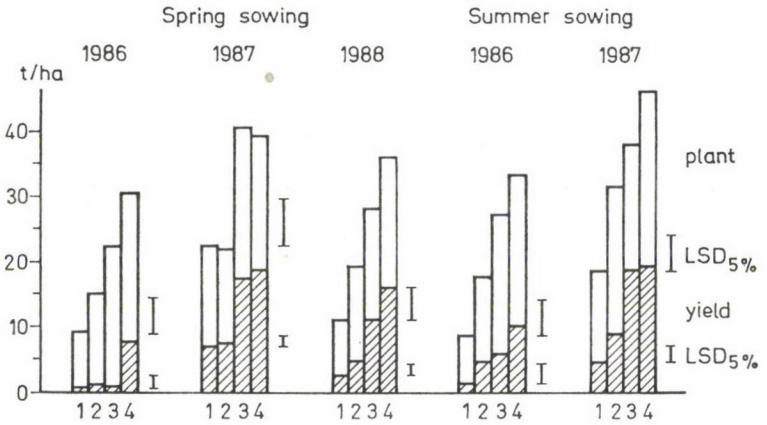

Fig. 1. The effect of different water supplies on the yield and plant bulk of snap bean. Variety: Valja, Gödöllő, 1986—1988

The results of our research concerning irrigation (Figure 1) show the insufficient quantity of precipitation in all the five growing periods where irrigation was ommitted. It is obvious that in the period of vegetative growth (treatment 2) irrigation increased the weight of foliage and plant, yet it was often insufficient and ineffective from the standpoint of yield. The normal plant yield weight — considering the presence of other natural conditions — was formed only if irrigation was regular, at least in the period of flowering and harvesting.

We also investigated the effect of the water supply on foliage temperature in the period of yield formation. Figure 2 shows the daily dynamics of 3 snap bean stands with different water supplies.

In the morning hours (6:00 to 7:00) the foliage temperature of plant stands with different water supplies was practically the same, and less than the air temperature. The temperature of stands with inadequate water exceeded the air temperature after 9 o'clock.

The unirrigated plant stand temperature was 2 to 3 °C higher than the air temperature in the middle of the day, which indicates that plants were unable to take up so much humidity from the soil that could have covered water demand for transpiration and other physiological processes (air temperature, radiation). The stand that had been irrigated with 40 mm dosage of water 8 to 9 days prior to measuring (treatment 2) showed that foliage temperature practically coincided with air temperature and this indicated the necessity for additional irrigation. The water supply of the stand irrigated with 40 mm of water a day before measuring (July, 5), (treatment 3) can be egarded as optimal, as is manifested by the formation of the foliage temperature, which was less than the air temperature during the day.

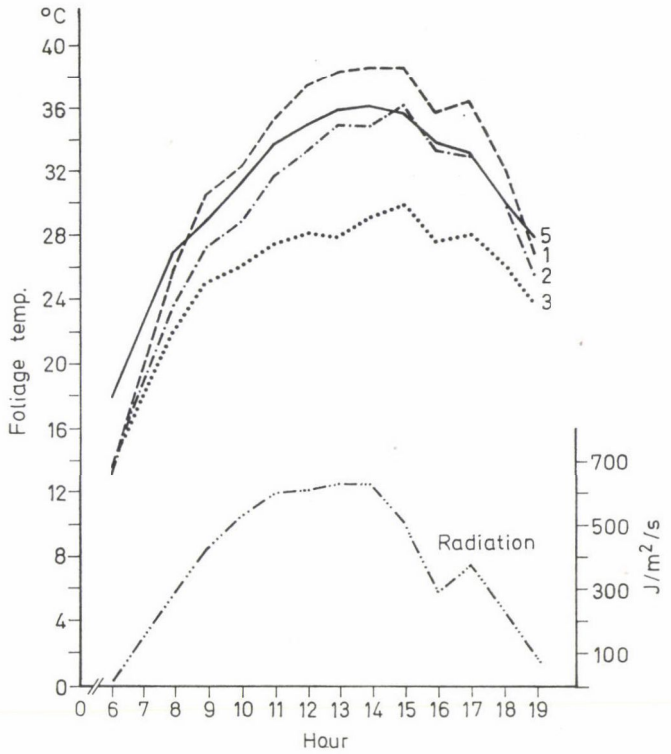

Fig. 2. The daily course of foliage temperature of snap bean stands with different water supplies Gödöllő, 1988. 6. 0.7—7.07 means

By early afternoon (13:00) when the air temperature was 36 °C with a radiation of 630 J/m²/s the foliage temperature of the unirrigated plant stock was 38.8 °C, the stock that just had started to indicate lack of water was 35.1 °C and the stand with an optimal water supply was only 28 °C.

The foliage temperature of snap bean stands with different water supplies was checked daily from the time prior to flowering until harvest. Figure 3 shows the results of measurements done during the spring of 1988. Unirrigated and regularly irrigated stands show the extreme dynamics specific to species. Since the beginning of flowering the water supply from the soil fails to satisfy the water demand of unirrigated stands if there is no precipitation, so insufficient transpiration cannot cool down the plants properly and the foliage temperature falls only after the air temperature falls.

In the case of an optimal water supply due to regular irrigation the snap bean is able to control its transpiration with regard to environmental conditions. Foliage temperature is often less than air temperature by some degrees, and seldom rises above 30 °C.

The foliage temperature of the stands irrigated in the two critical periods is very characteristic. First the foliage temperature of the stand irrigated before flowering was lower and the unirrigated (treatment 3) stand's temperature was higher. When the water supply in the soil was decreasing and irrigation was done as in the case of treatment 3, the lines of the diagram of the two stand cross, then treatment 3 can be regarded as optimal while treatment 2 reflects the effect of inadequate water (Figure 3).

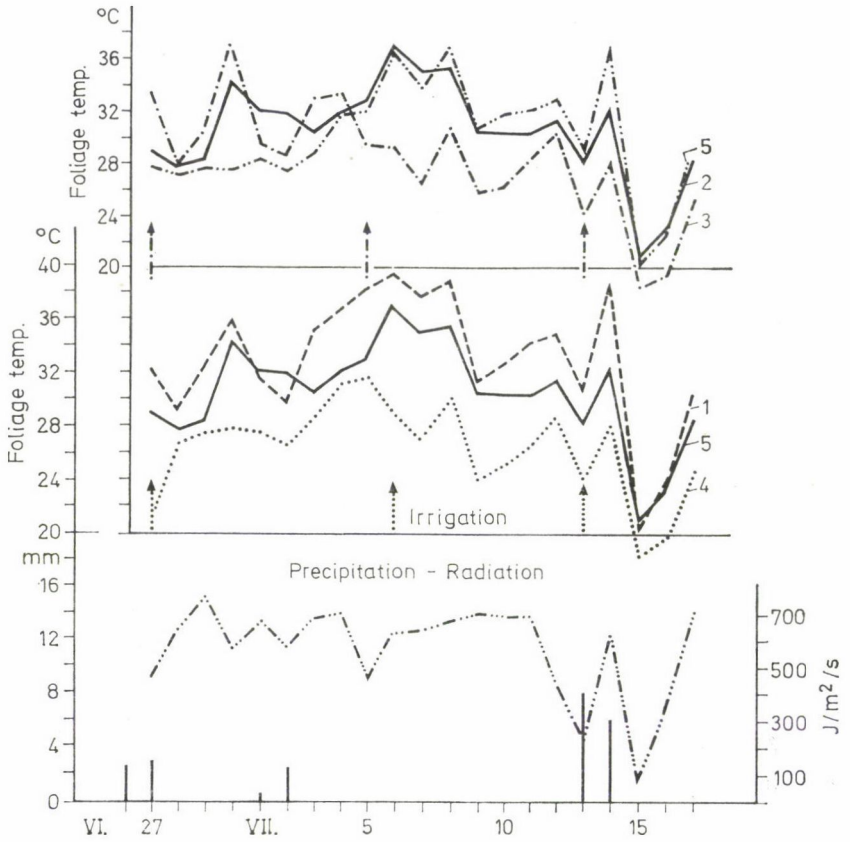

Fig. 3. The dynamics of foliage temperature at noon in the case of snap bean stands with different water supplies. Gödöllő, 1988

The course of the dynamics of foliage temperature in the case of the four different water supplies is well illustrated in Table 3. Here we demonstrate, besides the foliage temperature of the unirrigated stand, also the formulation of divergences in the treatments with regard to the LSD 5% values of daily variation analysis.

Sign "a" shows the highest temperature, "b" is significantly lower and "c" is significantly the lowest. The columns illustrate well the divergences in foliage temperatures in the case of the different treatments. Attention

Table 3

Significant divergences in foliage temperature of snap bean stands with different water supplies

Variety: Valja
Gödöllő, 1986. summer sowing

Day	Irrigation	Unirrigated foliage 1. temp. °C	$LSD_{5\%}$	1.	2.	3.	4.	F value significance
				treatments				
8.13.		26.3	—	a	a	a	a	—
14.		29.9	—	a	a	a	a	—
15.		28.2	1.1	a	b	a	b	××
16.		29.9	1.8	a	ab	a	b	××
17.		33.9	2.5	a	b	a	b	××
18.		35.5	2.0	a	a	a	b	××
19.	2.4.	34.1	1.0	a	b	a	b	×××
20.		23.6	2.1	a	b	a	b	+
21.		22.2	1.4	a	b	a	b	××
22.		26.1	2.0	a	b	a	b	××
23.		33.4	2.6	a	b	a	b	×××
24.		23.6	1.9	a	b	a	b	×
25.		23.5	2.7	a	b	a	b	×
26.		26.8	0.9	a	b	a	b	×××
27.	3.	32.8	2.4	a	b	b	b	×××
28.		36.2	1.1	a	b	b	b	×××
29.		13.2	—	a	a	a	a	—
30.		18.7	0.9	a	b	b	b	××
31.		24.8	1.4	a	b	c	bc	×××
9.01.		25.3	1.5	a	b	b	b	××
02.		22.3	—	a	a	a	a	—
03.		30.7	1.7	a	b	b	b	××
04.	4.	17.7	2.1	a	ab	b	b	+
05.		28.6	1.1	a	b	c	c	×××
06.		28.8	3.2	a	ab	bc	c	××
07.		30.7	1.9	a	b	c	c	×××
08.		32.2	1.3	a	a	b	c	×××
09.		35.4	1.7	a	a	b	c	×××
10.	3.	32.7	1.7	a	a	b	b	×××
11.		21.9	1.4	a	b	c	c	××
12.		32.4	2.1	a	a	b	b	×××
13.	4.	35.0	1.1	a	a	b	b	×××
14.		35.3	2.2	a	a	b	b	×××
15.		34.8	1.9	a	a	b	b	×××
16.		38.0	1.5	a	a	b	b	×××
17.		34.9	2.1	a	a	b	b	×××
18.		35.7	2.2	a	a	b	b	×××
19.		24.5	1.4	a	b	c	c	×××
20.		27.1	1.9	a	ab	b	b	××
21.		31.2	1.6	a	b	b	b	××
22.		21.8	1.2	a	a	b	b	×××
23.		28.2	1.3	a	a	b	b	×××

+, ×, ××, ××× F = 10, 5, 1, .1%

Table 4

Days of irrigation and average foliage temperature of snap bean stands with different water supplies

Gödöllő, 1986

Period	1.	2.	3.	4.	$LSD_{5\%}$	F value significance
	treatments					
	Days of irrigation					
Before flowering	—	8.04.	—	8.04.		
	—	8.19.	—	8.19.		
Mass flowering	—	—	8.27.	9.04.		
Legume formation	—	—	9.10.	9.13.		
	Average of foliage temperature at noon					
8.13—8.26.	28.4^a	25.8^b	28.6^a	25.4^b	.61	***
8.27—9.09.	27.0^a	24.6^b	23.3^c	22.7^c	.82	***
9.10—9.24.	31.0^a	29.8^b	25.7^c	25.4^c	.77	***

*** P = 0.1%

Table 5

Foliage temperature measured from the beginning of flowering of snap bean until harvest and the correlations of different parameters of plants (r values)

Variety: Valja

Gödöllő, 1986—1988

Indexes of foliage temperature (X)	$\bar{X}$	Spring sowing		
		Plant-	Foliage-	Yield-
		weight kg/ha Y		
	$\bar{Y}$	24604	16746	7857
Foliage temperatures				
— sum. > 0 °C	703	—.6438*	—.3151	—.7832**
— sum. > 10 °C	453	—.7775**	—.5058+	—.8509***
— sum. > 15 °C	329	—.8725***	—.6657*	—.8804***
— sum. > 20 °C	205	—.9364***	—.8064**	—.8751***
— maximum	36.9	—.9325***	—.7223**	—.9328***
— number of days above 30 °C	8.9	—.9508***	—.8929***	—.7722**
Differences of foliage and air temperatures				
— negative differences (foliage cooler than air)				
— sum.	—61.8	—.9240***	—.8067**	—.8552***
— extreme value	—6.7	—.7965**	—.7670**	—.6829
— number of days	16.3	.8447***	.8667***	.8355***
— positive differences (foliage warmer than air)				
— sum.	24.1	—.7901**	—.6594*	—.7544**
— extreme value	3.3	—.8588***	—.5561+	—.9417***
— number of days	8.7	—.8447***	—.8667***	—.8355***

***, **, *, + P 0.1, 1, 5, 10%

Number of evaluated operations — n = 12

Average number of days measured during operations: 25

must be paid as to how temperature changes with the time elapsed since irrigation. We can also observe the increasing divergence of foliage temperature values in the different treatments as vegetation time advances.

In regard to plant growth and time of irrigation, the data were divided into periods of 14 days. Table 4 illustrates the characteristic changes of foliage temperatures at noon.

Both the figures and the table prove the effect of irrigation on foliage temperature and well indicate the change of soil water supply. These data provide sound information on the irrigation requirement of the stands.

Our data reflect that the foliage temperature of snap bean stands gives a clear picture of their water supply. The daily water demand of plant is satisfied by the humidity available from the soil.

As we found a close correlation between water supply and yield, the question arose; what is the effect of foliage temperature, expressing the water supply of the plant, on the growth of plant and on its vegetative and generative parts and, furthermore, what is their correlation? Further we demonstrate our results in this respect.

We created two data groups for investigating the effect of foliage temperature on the plant:

(1) the actual values of foliage temperature,

(2) foliage temperature differing from air temperature.

The period from the beginning of flowering until harvest was the most characteristic in the case of both groups (it was 21—32 days in our experiments).

Table 5 is the best for the characterization of the correlation between plant and yield quantity:

- the sum of foliage temperatures, but a more exact result is given by the active degree days expressing the duration of period and the heat demand of snap bean,
- The maximum of foliage temperatures shows a close correlation (this value shows the date of one day, but its formulation is the end result of the process following the decrease of soil water supply).
- It is very interesting that the number of days proved to be very good for the characterization of the investigated period and for the summary of the difference in water supply, when foliage temperature at noon exceeded 30 °C.

In fact we have found similar correlations during the evaluation of differences in air and foliage temperature; the summary of foliage temperatures warmer or colder than air, the extreme values and their frequency well express the water and heat supply of plants.

Correlation investigations indicate the connection between foliage temperature and the plant production. So far we have pointed out only a linear

correlation. A greater number of data will give a more exact evaluation of the character and description of correlation.

If we consider only the closer correlations, reliable on P = 0.1% level, we can conclude that foliage temperature shows close correlation

- with the total plant products, biomass,
- a marked correlation with the amount of yield,
- correlation is naturally less with foliage weight having formed during the investigated period. The probability is that foliage is destroyed and decreases because of water deficiency.

We have proved that high air temperature is very favourable for the formation of yield and plant weight in the case of snap bean that has a great demand for humidity and heat. In other words, sufficient water supply ensures greater production.

In the case of undisturbed transpiration, the plant is not overheated even during the warmest hours and assimilation is normal.

In the case of water deficiency, the yield depression is stronger if the foliage temperature exceeds 30 °C; the higher the foliage temperature, the weaker the yield formation.

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RESPONSE OF MAIZE TO THREE NITROGEN SOURCES WITH AND WITHOUT NITRAPYRIN

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Five field experiments were conducted at Gödöllő and Hatvan agricultural experimental stations, University of Agricultural Sciences, Gödöllő, Hungary, during the three successive seasons of 1985, 1986 and 1987 to discover the effect of three nitrogen sources (Formurin, urea and ammonium nitrate) with and without nitrapyrin on yield and quality of maize.The most important findings could be summerized as follows:

— The weight per hundred kernels was significantly affected by nitrapyrin in both seasons. The obtained increment due to nitrogen carriers was marked in the first season.

— Usage of nitrapyrin satisfactorily improved the crude oil percentage in grains in both seasons.

— Nitrapyrin favourably improved the crude starch percentage in grain in the two seasons. In both seasons, the three nitrogen carriers improved this character, and this improvement was marked in the second season.

— Crude fiber percentage in grains was satisfactorily increased by all the studied treatments in both seasons.

Nitrapyrin increased the grain yield per plant grown at Gödöllő and Hatvan and this increment was significant in the first season at Gödöllő. The three nitrogen sources, also increased this trait at Gödöllő and Hatvan, and this was appreciable at the first location at Hatvan.

— Grain yield per ha was markedly affected by nitrapyrin in the first season at Gödöllő and Hatvan. Nitrogen carriers magnified this attribute over all the experiments and the highest grain yield per ha was produced by the addition of ammonium nitrate in three equal parts.

The highest grain yield and quality of maize resulted from the addition of nitrapyrin at the rate of 4 l/ha with nitrogen fertilizer in an ammonium nitrate form at the rate of 150 kg N per ha in three equal portions added at preplanting, at knee height stage and at tasseling stage.

Keywords: maize, Zea mays L., nitrogen sources, Nitrapyrin application, plant response, yield parameters.

Introduction

Maize (*Zea mays* L.) is one of the major world-wide agronomic crops. The importance of maize as a main food source for mankind has increased especially in the developing countries to selve the world's food problem. In Egypt the productivity of corn grain yield is relatively low, even under

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the best environmental conditions for corn production. Two of the principal reasons for low productivity of maize in Egypt is nitrogen and water management. The use of nitrification inhibitors as a nitrogen management tool has increased in recent years. Much of the research involving inhibition of nitrification has been conducted using nitrapyrin (2-chloro-6-trichloromenthyl-pyridine). Boswell et al. (1974), mentioned that the grain yield of corn was increased by 14% due to nitrapyrin addition compared with no nitrapyrin. Warren et al. (1975) indicated that nitrapyrin increased the 100 kernel weight, protein yield in grains and grain yield per ha. of corn. Several results are reported by Malzer et al. (1979) and McCornick et al. (1984).

In addition to the quantity, the time and source of nitrogen fertilizer are important factors for improving corn production. When nitrogen fertilizer is applied to a soils, its utilization is limited as a result of fixation by soil and/or losses through leaching, denitrification and volatilization. One of the best ways of increasing the utilization of added nitrogen by the plants is to time the nitrogen applications so as to synchronize them with the crop demand. The purpose of this study was to determine the effects of three nitrogen fertilizers with and without nitrapyrin on yield and quality of corn grown under Hungarian conditions.

Material and methods

These investigations were conducted during the three successive growing seasons of 1985, 1986 and 1987 to study the effect of nitrogen sources with and without nitrification retarder nitrapyrin on yield, yield components and some quality traits of Maize (*Zea mays* L.). Therefore, five field experiments were carried out at the agricultural experimental station of the University of Agricultural Sciences, Gödöllő, Hungary. The first year involved an attempt at Gödöllő; the second year included three trials, one at Gödöllő and two at Hatvan at two different locations; the third year involved one experiment at Hatvan to confirm the effect of the studied factors on corn grain yield. The variety used was "Pioneer 3747 SC" (FAO-400) and the experimental soil was sandy and loamy at Gödöllő and Hatvan, respectively. The second year of study was drier than the first and the third year. Maize grains were sown during the first half of May at the rate of 70,000 grains per ha with 20 cm distance between each successive plant.

Corn grains were harvested during the first half of October. The number of harvested plants and number of ears were recorded for each plot at harvesting time. The moisture content of grain at harvesting time was measured by electronic moisture meter (Protimeter).

A strip plot design, with four replicates and fourteen treatments, was used as follows:

A — The vertical-strip plots: included 2 treatments of nitrapyrin and untreated control.
- 1 — Untreated control
- 2 — Nitrapyrin at 4 l/ha.

B — The horizontal strip plots: included 7 treatments of nitrogen fertilizers.
- 1 — Unfertilized control
- 2 — Formurin in single addition
- 3 — Formurin in split addition
- 4 — Urea in single addition
- 5 — Urea in split addition
- 6 — Ammonium nitrate in single addition
- 7 — Ammonium nitrate in split addition

Nitrapyrin was applied immediately prior to sowing time, then incorporated within the soil.

N fertilizers were added at a rate of 150 kg N/ha either in full dose preplanting or in three equal portions: at planting time, at knee height stage and at tasseling stage.

The experimental unit contained four rows each, five meters in length and seventy cm distance between each successive row. At harvest, ten ears were taken at random from each sub-plot for the determination of yield components which comprised: ear length (cm), ear diameter (cm), 100-kernel weight (adjusted to 15.5% H_2O). All plots were harvested and grain yield was determined and connected at 15.5% H_2O. Grain quality parameters were measured by INFRAPID[61] equipment. The data from each experiment were analyzed according to Gomez and Gomez (1984).

Results and discussion

Ear size (length and diameter cm)

It could be seen from Table (1) that both ear length and ear diameter significantly and insignificantly increased by the treatments of nitrapyrin in the first and second season, respectively. The first year was wet while the

Table 1

Average ear length (cm) and ear diameter (cm) as affected by nitraphyrin, nitrogen source and time of addition in 1985 and 1986 seasons

Treatment	Ear length (cm)			Ear diameter (cm)		
	1985	1986	mean	1985	1986	mean
N. I. Agent						
Untreated	16.11	17.07	16.59	4.43	2.53	3.48
Nitrapyrin 4 l/ha	19.18	17.62	18.40	4.50	2.43	3.46
F. test	**	N. S		*	N. S	
LSD 5%	0.579	—		0.067	—	
N. sources: 150 kgN/h						
Unfertilized	17.44	17.18	17.31	4.40	2.30	3.35
Formurin: single addi.	18.32	17.77	18.04	4.47	2.41	3.44
Formurin: split addi.	18.52	18.25	18.38	4.53	2.42	3.47
Urea: single addi.	18.36	18.37	18.36	4.47	2.44	3.45
Urea: split addi.	18.02	17.12	17.57	4.50	2.36	3.43
Am. nitrate: single addi.	18.20	17.25	17.72	4.51	2.38	3.44
Am. nitrate: split addi.	18.46	17.75	18.10	4.53	2.37	3.45
F. test	N. S	N. S		N. S	N. S	
LSD 5%	—	—		—	—	

second year was relatively dry. In connection with the effect of nitrogen carriers and their time of application on both ear length and ear diameter in both seasons, it was quite clear that the two parameters were not influenced by these treatments. These results agree with those obtained by Faisal (1983), Gascho and Hook (1984) and Ali (1985).

100-kernel weight (g)

In both seasons 100 kernel weight was increased by nitrapyrin, as indicated in Table (2). These treatments had marked influences on this par-

Table 2

Average 100 — Kernel weight (gm) and shelling percentage as affected by nitrapyrin, nitrogen source and time of addition in 1985 and 1986 seasons

Treatments	100 — Kernel weight (gm)			Shelling percentage		
	1985	1986	mean	1985	1986	mean
N. I. Agent						
Untreated	29.09	23.91	26.50	84.94	84.95	84.94
Nitrapyrin 4 l/ha	29.40	25.91	27.65	84.85	84.25	84.55
F test	*	**	N. S		N. S	
LSD 5%	1.556	1.135		—	—	
N. sources: 150 kg N/h						
Unfertilized	27.03	23.55	25.29	85.24	85.34	85.29
Formurin: single addi.	29.54	26.04	27.79	84.59	85.05	84.67
Formurin: split addi.	29.97	25.08	27.52	84.57	84.92	84.74
Urea: single addi.	29.57	25.29	27.43	84.95	84.51	84.73
Urea: split addi.	30.06	24.75	27.40	84.77	84.85	84.81
Am. nitrate: single addi.	30.51	24.99	27.75	84.47	84.57	84.52
Am. nitrate: split addi.	29.83	24.41	27.12	84.98	84.53	84.75
F. test	*	N. S		N. S	N. S	
LSD 5%	2.058	—		—	—	

ameter and remained constant in the two seasons. The nitrapyrin effect may be explained by the fact that the use of nitrapyrin reduced leaching and denitrification, permitted addition without loss, and extended the response to avoid a sudden growth, thus influencing the 100-kernel weight. These results are supported by those of Rajal and Prasad (1974) and Warren et al. (1975).

The relevant data revealed that the three nitrogen sources, namely, formurin, urea and ammonium nitrate significantly and insignificantly increase 100-kernel weight in the first and second reason respectively, as compared with the control. Over the two seasons, it can be seen that ammonium nitrate was superior to urea. These results agree with those of Ali (1985).

Shelling percentage

Table (2) indicates that all the studied factors did not affect in any season the average 100-kernel weight. It seems that this attribute is a genetic character which is less affected by the environmental factors. These results are in line with those obtained by Sprague et al. (1977), Gascho and Hook (1984) and Ali (1985).

Crude protein percentage

As indicated by Table (3), the addition of nitrapyrin had no marked effect on the character of crude protein percentage in corn grain and that

held true in the two seasons. Similar results were reported by Warren et al. (1975), Tsai et al. (1978) and Maddux et al. (1985).

Table 3

Average crude protein percentage and crude oil percentage in grains as affected by nitrapyrin, nitrogen source and time of addition in 1985 and 1986 seasons at Gödöllő

Treatments	Crude protein percentage			Crude oil percentage		
	1985	1986	mean	1985	1986	mean
N. I. Agent						
Untreated	10.41	10.06	10.23	4.55	4.48	4.51
Nitrapyrin 4 l/ha	10.48	10.26	10.37	4.73	4.78	4.75
F. test	N. S	N. S		**	**	
$LSD_{5\%}$	—	—		0.08	0.11	
N. sources: 150 kg N/h						
Unfertilized	9.85	9.88	9.86	4.51	4.54	4.52
Formurin: single addi.	10.35	10.27	10.31	4.63	4.64	4.63
Formurin: split addi.	10.69	10.26	10.47	4.65	4.62	4.63
Urea: single addi.	10.83	10.35	10.59	4.69	4.74	4.71
Urea: split addi.	10.52	10.53	10.52	4.61	4.68	4.64
Am. nitrate: single addi.	10.55	10.48	10.51	4.58	4.72	4.65
Am. nitrate: split addi.	10.59	10.33	10.46	4.67	4.58	4.62
F. test	**	N. S		N. S	N. S	
$LSD_{5\%}$	0.500	—		—	—	

The relevant data revealed that the three nitrogen fertilizers significantly and insignificantly increased the crude protein percentage in corn grain in the first and second season, respectively. Over the two seasons, urea was the most efficient nitrogen source, followed by ammonium nitrate and then formurin. The nitrogen effect may be due to the fact that nitrogen is essential for building up the protoplasm, amino acids and consequently proteins. These findings are supported by the result of Taber and Cox (1983).

Crude oil percentage

Data presented in Table (3) show that crude oil percentage in corn grain was satisfactorily improved by nitrapyrin in the two growing seasons. These results are in harmony with those of Tsai et al. (1978).

The relevant data revealed that the three nitrogen carriers had no significant effect on this attribute in either season. This agrees with the results of Tsai et al. (1978).

Crude starch percentage

It is obvious from Table (4) that crude starch percentage in corn grain was significantly and insignificantly affected by the treatment of nitrapyrin

Table 4

Average crude starch percentage and crude fiber percentage in grains as affected by nitrapyrin, nitrogen source and time of addition in 1985 and 1986 seasons at Gödöllő

Treatment	Crude starch percentage			Crude fiber percentage		
	1985	1986	mean	1985	1986	mean
N. I. Agent						
Untreated	63.43	63.09	63.26	4.23	4.75	4.49
Nitrapyrin 4 l/ha	63.63	63.21	63.42	4.81	5.04	4.92
F. test	**	N. S		**	**	
$LSD_{5\%}$	0.24	—		0.18	0.14	
N. sources: 150 kg N/h						
Unfertilized	63.54	62.82	63.18	4.25	4.70	4.47
Formurin: single addi.	63.79	63.39	63.59	4.72	4.93	4.82
Formurin: split addi.	63.70	63.53	63.61	4.64	4.94	4.79
Urea: single addi.	63.63	63.02	63.32	4.58	5.11	4.84
Urea: split addi.	63.68	63.30	63.49	4.58	4.98	4.78
Am. nitrate: single addi.	63.77	63.24	63.50	4.49	5.16	4.82
Am. nitrate: split addi.	63.67	63.42	63.54	4.65	5.07	4.86
F. test	N. S	*		**	**	
$LSD_{5\%}$	—	0.40		0.24	0.18	

in the first and second season, respectively. In both seasons, this trait was improved by these treatments. This results are in line with those of Tsai et al. (1978).

The data also showed that all the three nitrogen sources increased this attribute over the control in the following order: Formurin > ammonium nitrate > urea (over the two seasons). The increase was insignificant and significant in the first and second reason, respectively. These results are in harmony with those of Tsai et al. (1978).

Crude fiber percentage

In both seasons the plants receiving nitrapyrin showed a satisfactory increase in crude fiber percentage, compared with the untreated plants. Moreover, it could be clearly seen that this trait increased markedly by the three nitrogen sources over the control in the two seasons in the following order: ammonium nitrate > urea > formurin.

Grain yield per plant (g)

Under Gödöllő conditions, plants receiving nitrapyrin produced a marked increase in grain yield per plant in the first and second season, if compared with the control as shown in Table (5). These results may be due to the favour-

Table 5

Average grain yield per plant (gm) as affected by nitrapyrin, nitrogen source and time of addition in both seasons at Gödöllő and at two locations at Hatvan

Treatments	Gödöllő			Hatvan		
	1985	1986	mean	1986 site I	1986 site II	mean
N. I. Agent						
Untreated	124.25	120.14	122.19	51.35	36.10	43.72
Nitrapyrin 4 l/ha	149.32	124.60	136.96	54.32	43.07	48.69
F. test	**	N. S		*	N. S	
$LSD_{5\%}$	9.714			4.171	—	
N. sources: 150 kg N/h						
Unfertilized	133.31	113.00	123.15	44.25	31.37	37.81
Formurin: single addi.	141.62	118.43	130.02	58.75	36.50	47.62
Formurin: split addi.	145.18	127.68	136.43	55.75	41.68	48.71
Urea: single addi.	142.06	129.81	135.93	55.93	36.06	47.99
Urea: split addi.	141.12	128.81	134.96	55.75	38.12	46.93
Am. nitrate: single addi.	146.12	122.68	134.40	54.06	40.18	47.12
Am. nitrate: split addi.	146.87	127.43	137.15	54.18	43.62	48.90
F. test	N. S	N. S		**	N.S	
$LSD_{5\%}$	—	—		5.518	—	

able conditions for nitrogen losses in the first season compared with the second season. The plants grown under Hatvan conditions were significantly and insignificantly affected by these treatments in the first and second location, respectively. These data are in line with those of Jauert et al. (1968).

It can be also seen from Table (5) that nitrogen sources had no marked effect on grain yield per plant in either season at Gödöllő and in the second site a Hatvan. This trait was increased by the three nitrogen fertilizers in the first location at Hatvan. In general, the three nitrogen sources increased this trait over the control in both Gödöllő and Hatvan experiments in the following order: ammonium nitrate urea > formurin. In all of the trials carried out at Gödöllő and Hatvan, ammonium nitrate added in three equal portions produced the maximum average grain yield per plant. Similar results were found by Muirhead et al. (1985).

Grain yield per ha (t)

Data in Table (6) show that grain yield per ha increased considerably and inconsiderably at Gödöllő area in the first and second season, successively, by the addition of nitrapyrin. This effect may be explained by the same fact mentioned with respect to grain yield per plant. These treatments had a significant and insignificant influence on this trait at Hatvan area in the first and second season, respectively. Generally, nitrapyrin improved grain yield

Table 6

Average grain yield per hectar (ton as affected by nitrapyrin, nitrogen source and time of addition in both seasons at Gödöllő and Hatvan

Treatments	Gödöllő			Hatvan			
	1985	1986	mean	1986 site I	1986 site II	1987	mean
N. I. Agent							
Untreated	6.84	7.21	7.02	4.93	3.98	5.50	4.80
Nitrapyrin 4 l/ha	8.70	7.48	8.09	4.96	4.61	5.03	5.20
F. test	**	N. S		**	**	N. S	
$LSD_{5\%}$	0.422	—		0.368	0.356	—	
N. sources: 150 kg N/h							
Unfertilized	8.02	6.71	7.36	4.23	3.73	4.72	4.22
Formurin: single addi.	8.19	7.55	7.87	5.47	4.16	6.14	5.25
Formurin: split addi.	8.16	7.58	7.87	5.04	4.39	5.88	5.10
Urea: single addi.	7.66	7.76	7.71	5.60	4.15	5.64	5.13
Urea: split addi.	7.84	7.52	7.68	5.27	4.31	6.05	5.21
Am. nitrate: single addi.	7.80	7.37	7.58	5.15	4.66	6.20	5.33
Am. nitrate: split addi.	8.31	7.83	8.07	5.22	4.84	6.38	5.48
F. test	N. S	N. S		**	**	**	
$LSD_{5\%}$	—	—		0.487	0.471	0.750	

per ha over the control in all the trials at both Gödöllő and Hatvan. These results are in harmony with those of Boswell et al. (1974), Warren et al. (1975), Malzer et al. (1979) and McCormick et al. (1984).

The relevant data revealed that this character was not influenced by the three nitrogen carriers in the two seasons at the Gödöllő area. In both seasons at Hatvan, grain yield per ha was generally increased by the three nitrogen sources. In general, all nitrogen carriers increased average grain yield per ha over the control, and the most efficient nitrogen source was ammonium nitrate added in three equal section at planting time, at knee height stage and tasseling stage. The superiority of split addition to the single addition may be attributed to the possibility that when nitrogen was added in full dose at one time, N might have leached down because of the inability of the young plants to utilize it fully; but when nitrogen was applied in splits, the plant was able to utilize more nitrogen because it was available in smaller doses over a longer period. Similar results were reported by Volk (1966), Fox and Hoffmann (1981), Touchton and Hargrove (1982) and Fox et al. (1986).

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BORON CONTENT OF LUCERNE

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Lucerne samples, 188 in number, obtained from large-scale farms contained an average of 43.0 ± 12.8 mg/kg boron at the beginning of flowering. Since on the basis of 652 samples of 83 species analysed by the author the family of *Papilionaceae* takes up 27.4 mg/kg boron on an average, *Medicago sativa* has in any case an outstanding boron content. The spontaneously producing *Medicago* species are also of high boron concentration. According to the author's original analyses in Hungary, an average of 25.4 ± 8.7 mg/kg boron was contained in over 7000 samples of more than 1000 species. In a number of measuring series the boron uptake of lucerne went parallel with the uptake of calcium and magnesium. This relationship is probably characteristic of the whole living world and is of a biochemical nature. The relation of boron and manganese observed in the fertilization- and liming experiments is the joint result of the chemical reaction of soil. With liming a decrease in boron uptake unfavourably affecting the volume of yield may occur. The B-Na relation observed follows the changes in the concentration of the two parallel accumulating and highly water soluble elements.

Keywords: boron, ion relations, chemotaxonomy, lucerne, fertilization.

Introduction

After wheat and maize lucerne occupies the largest arable area — some 400.000 ha — in Hungary. In spite of the both plant physiology (Frenyó and Márton 1958) and farm-scale experiments (Kuthy, Mérei, Varsányi 1953, Leszek 1964, Pekáry 1970) conducted with the boron supply of lucerne, boron analyses have not been published. None of the 160 papers written by Hungarian authors in the period of agricultural expansion dealt with the question (Anonym 1984). Since the lucerne plant requires much boron, and through, it the boron has an influence on the metabolism of animals (Bussler 1968. Kovalszkij 1974, Rigó et al. 1986), it was necessary to make a wider survey. It seemed to be important to clear the chemotaxonomic role of boron, because according to earlier observations (Tölgyesi 1965) in the microelement uptake of plants their taxonomic place is of decisive importance, and the earlier works mostly dealt with metallic elements only. In the present paper the boron content of the cultivated lucerne, and out of the factors influencing it some fertilizer- and soil effects are discussed. Further, it is compared for

boron content with the family *Papilionaceae* and with 85 plant families of the Hungarian flora.

Materials and methods

The material presented here was collected from commercial lucerne fields of Hungary. The series discussing the fertilizer effects originate from experiments organized first of all by Pekáry; their evaluation from a different aspect has been made in the form of manuscript. The samples were scissored off 3 cm above ground level at the beginning of flowering, then dried, ground, and 5 g of each measured in for the purpose of determination. Before reduced to ashes the material was wetted with 10 ml aqueous suspension containing 50 mg $Ca(OH)_2$ to prevent the loss of boron. The ash was dissolved in 0.2 normal hydroehloric acid and the boron determined by colorimetry with the method of Hatcher and Wilcox (1950). The other elements were measured by atom absorption and spectrophotometry.

Results

The 188 lucerne samples contained 43.0 mg/kg boron on an average, which almost exactly agrees with the median (42.9). The standard deviation is 12.8, the variation coefficient 0.297, the distribution nearly normal. The latter is indicated by the negligible rightwise skewness (mom. coeff. of skewness: 0.15) and also the mild peak (mom. coeff. of kurtosis: 3.30) of distribution (Fig. 1).

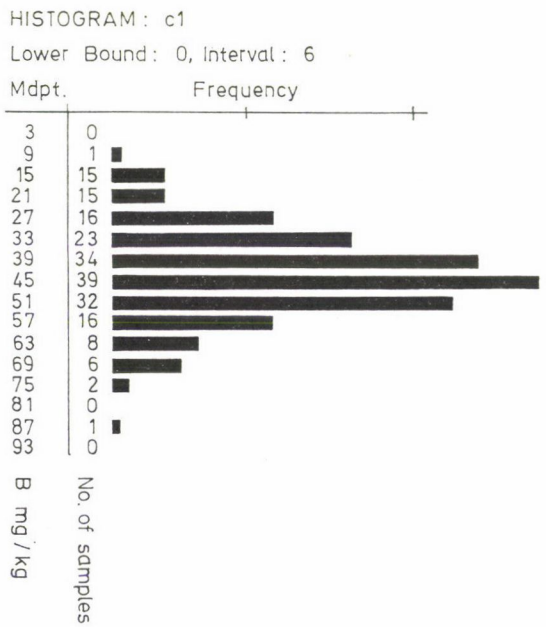

Fig. 1. Distribution of boron content of 188 lucerne samples

With 12 other elements in addition to boron included in the calculations, 46% of the change of boron could be explained by the change of the other elements. Sodium and aluminium equally with 10.9% and potassium with 6.5% shared the effect. Among the simple linear relations the boron-magnesium relation was significant ($r = 0.36$), and so were the boron-sodium and boron-aluminium relation, both characterized with a correlation coefficient of 0.27 ($n = 57$). The boron-magnesium relation is shown in Fig. 2.

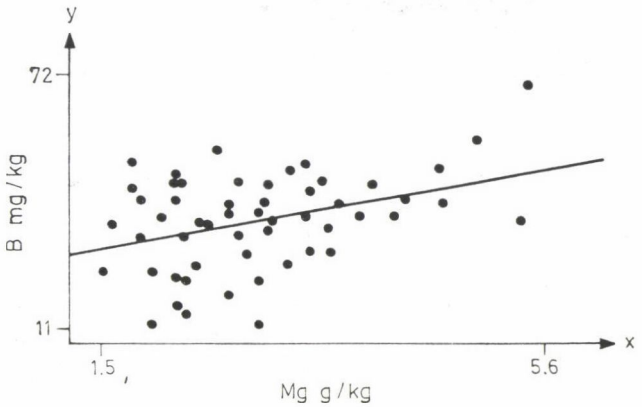

Fig. 2. Relationship between magnesium and boron content of 57 lucerne samples

Soil- and fertilizer effects

In field experiments treated with fertilizer containing varying amounts of NPK and with calcium carbonate 90 samples were analysed. The effects of the different fertilizers will be discussed on another occasion, only the variation produced under the conditions of practice are shown here: B 37.9 $\pm$ $\pm$ 3.9, Mn 80.2 $\pm$ 24.8, Zn 30.3 $\pm$ 9.4, Al 209 $\pm$ 151, Mo 0.53 $\pm$ 0.27. While the different interventions influenced the aluminium to 72%, and the Mo to 51% (the error of the molybdenum analysis is only 5%), the zinc and the manganese could be characterized equally by a variation of 31%, and the variation of boron was of a mere 10% value. The boron content that we are interested in was in a significant negative correlation ($r = -0.21$) with the molybdenum content, and in a significant positive correlation ($r = 0.61$) with the manganese content. The latter is shown in Fig. 3.

Owing to its practical importance the boron treatment is often combined with molybdenum treatment. In an experiment boron and molybdenum were supplied each on three rising levels to two soils. The boron contents of the 24 samples analysed were unusually high, 66.5 $\pm$ 8.5 mg/kg, while the concentrations of the other 10 elements were all in negative correlation with the boron content, and some of them were significant: K = -0.82,

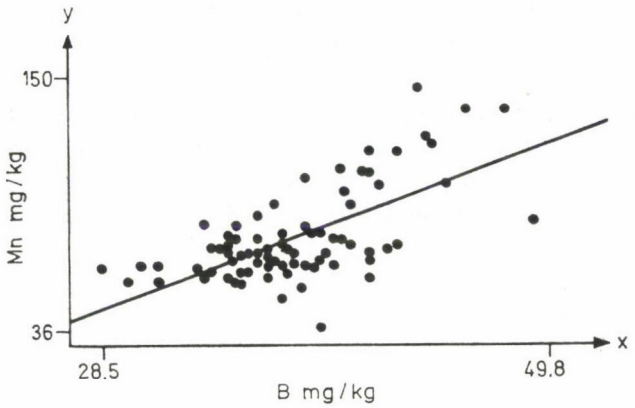

Fig. 3. Relationship of boron and manganese content in a fertilization experiment ($n = 90$)

Mg = −0.71, P = −0.68, Cu = −0.61 and Ca = −0.61. In this series of measuring the change of the boron content was explained to 83% by the change of the other elements, and on the basis of the multiple regression to 36.3% by the change of potassium and to 30.6% by that of magnesium.

In a culture pot experiment the boron content was 58.3 ± 9.8 mg/kg, and the molybdenum content 2.69 ± 2.22 mg/kg at the time of sampling. The experiment was set up on 8 soil types with ∅, Mo, Mo + B combinations. The boron content in the lucerne was in significant correlation with the Ca content ($r = 0.55$), the phosphorus content ($r = 0.53$) and the Mn content ($r = 0.50$). The Ca-B correlation is to be seen in Fig. 4. The average boron concentration of lucerne grown on the 8 soils on the average of the three treatments is: Putnok 46, Mucsony 48, Szilvásvárad 49, Cered 50, Czirák 51, Pétervására 59, Bogács 64 and Pásztó 71. The significant difference is 15 mg/kg.

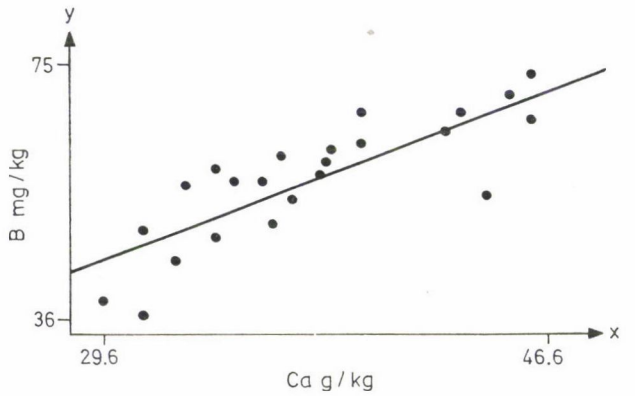

Fig. 4. Relationship of calcium and boron content in a B- and Mo fertilization experiment made on eight soil types in a culture pot ($n = 24$)

In the field experiments with varying Ca-, NPK- and Mo nutrition on two soil types 44.2 ± 11.7 mg/kg boron content was obtained on the average of 30 samples. The change in the boron content of lucerne could be brought into connection to 85% with the change of the other elements examined. Ca (+29.2%) and Mg (−28.2%) could be proved to have the greatest effect, at the same time the simple linear correlations between boron and calcium and boron and magnesium can be characterized with correlation coefficients of 0.44 and −0.36, respectively.

The analysis of 60 samples from three cuttings for the effect of lime, NPK and Mo on a soil at Putnok pointed out an average of 34.5 ± 5.1 mg/kg boron content. On the basis of multiple regression, 54% of the change in the boron content can be explained by the change of the other 10 elements examined, above all by the change of potassium: to 26.2%. According to the linear correlations, a significant connection existed with the uptake of Ca ($r = 0.32$), P ($r = 0.32$) and Mg ($r = 0.26$) too.

On eight soils 43.4 ± 5.6 mg/kg boron concentrations were obtained in response to ∅, B and Mo treatments. Against boron a number of significant correlations could be established: Mg 0.72, Ca 0.70, Fe 0.58, Mn 0.57 and Na 0.43. On the basis of the multiple regression the change in the boron uptake could be explained to 70 % by changes in the other 10 elements. Out of it magnesium was responsible for 33.5%, while in this case the share of calcium was a mere 3.6%. The linear B-Mg correlation is shown in Fig. 5.

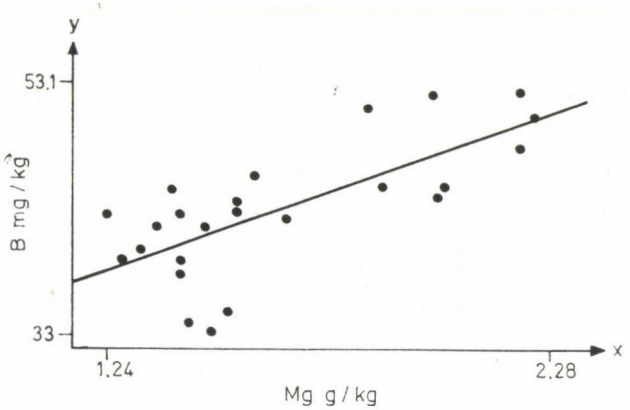

Fig. 5. Relationship of magnesium and boron in a B- and Mo fertilization experiment made on eight soil types ($n = 24$)

Discussion

The average 43 mg/kg boron content of lucernes in Hungary can be categorized in comparison with the boron contents of other plants grown under the same climatic and soil conditions. First its place in the family of

Papilionaceae must be taken into considerations (Table 1). In 43 samples of the 4 spontaneously producing *Medicago* species examined, the boron content was 41.0 mg/kg, hardly different from the boron content of the cultivated *Medicago sativa*. However, the genus is a representative of the family that takes up the largest amount of boron. Lucerne is even more outstanding when

Table 1

Boron content in more frequently analysed genera of the family Fabaceae in terms of mg/kg dry matter

Genus	Species number	Sample number	$\bar{x}$	s	CV
Amorpha sp.	1	28	26.2	10.3	38.3
Astragalus sp.	6	17	20.6	1.9	9.2
Coronilla sp.	1	21	24.3	8.7	35.8
Cytisus sp.	5	13	39.5	20.4	51.6
Genista sp.	4	10	23.1	9.2	39.8
Lathyrus sp.	11	119	22.3	3.1	13.9
Lotus sp.	4	65	39.1	11.8	30.2
Medicago sp.	4	43	41.0	2.9	7.1
Melilotus sp.	2	31	28.4	0.4	1.4
Robinia sp.	1	57	33.6	16.7	49.7
Trifolium sp.	17	187	25.8	4.3	16.7
Vicia sp.	14	50	26.0	10.3	39.6

we consider that in 652 samples of 83 species from the family *Fabaceae* examined by us only 27.4 mg/kg boron was measured.

When considering the distribution of boron content in 85 plant families including more than 1000 species, as determined in more than 7000 analyses (Fig. 6) we find the boron-accumulating character of lucerne even more

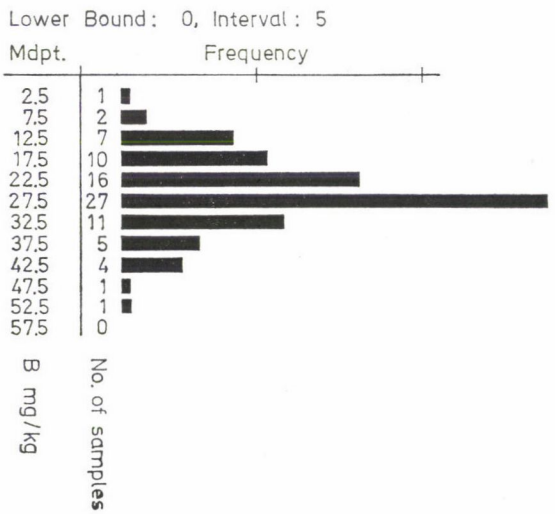

Fig. 6

conspicuous. The B average of the plants is 25.4 ± 8.7 ppm, and only two families have a higher than 45 ppm average boron content. This high capacity of boron uptake must be reckoned with when planning the nutrient replacement in crop production. On the other hand, the high boron content of lucerne calls attention to the possibility that, through its influence on ion antagonisms and enzyme activities, decreased performance or diseases may occur (Tölgyesi 1989).

The boron content of lucerne is influenced by external factors (soil, fertilization etc.) too. The scatter of boron contents in lucerne samples collected from various types of soil (n = 188 and 57) is equally near 30%. In the briefly described fertilization experiments the range was 10.3—327.7. This variation is not insignificant either, but the extreme cases occurring cause practical problems from the side of both deficiency and excess.

Among the correlations between boron and other elements the B-Ca correlation appears to be the most important; it was significantly positive in two field- and two culture pot experiments. The presence of this parallel within a species confirms the phenomenon described for different taxonomic units, e.g. grasses and papilionaceous plants (Tölgyesi and Kozma 1974). The high Ca- and B content of Fabaceae diverges widely from the equally low values of Gramineae.

The second, frequently observed correlation of B-Mg was positive in field experiments with 57 and 60 samples, respectively, and in a culture pot experiment with 24 samples. This correlation can be observed in the metabolism of the animal organism too, and probably is felt through a boron-magnesium-calcium action series. That the positive Mg-B correlation is an action of the soil is demonstrated by the fact that the hot water-soluble boron content and the soluble Mg content are in correlation in the soil (Tölgyesi and Kozma 1974). The only negative B-Mg correlation occurred in a series where the concentration of each element was reduced by the seldom observed high B concentration. This seems to be due to an excess of boron. The same conclusion was drawn by Miller and Smith (1977) who with ∅, 6.3, 12.6 kg/ha rate of boron nutrition obtained decreasing concentrations for all the 11 elements (Zn, Fe, Mn, Mg, Ca, P, K, Na, Al, Si, Cu) parallel to the increasing B concentrations.

The B-Mn correlation established in 90 samples from the field experiment mentioned, and in 24 samples from each of two culture pot experiments can be reliably interpreted. The difference in the original soil properties on the one hand, and the changes in pH caused by liming, on the other, influenced the availability of both elements in the same way. In culture fluid experiments carried out by Fox (1968) changes in the chemical reaction did not influence the boron uptake of lucerne. In the experiments of Tölgyesi and Kozma (1974), however, a negative correlation was found between the chemical

reaction and the boron uptake of plants. This was confirmed by Keresztény (1972) who in 122 soils established a —0.40 value of correlation between the pH and the soluble B content. In the experiments conducted by Palkovics and Győri (1984) lime added to the NPK fertilizer decreased the B uptake of potato. The importance of the chemical reaction in the case of Mn and Mo was also suggested by our earlier analyses (Tölgyesi and Kozma 1967), while for nitrogen it was indicated by Pekáry, Mártonffy and Sulyok (1976). To changes in the chemical reaction of the soil, most plants, lucerne in particular, respond with a change in the ratio of Mn/Mo; namely, with a decreasing pH this ratio increases. Since both the molybdenum and the boron are microelements of vital importance, care must be taken not to reduce the boron content in an unfavourable measure by supplying calcium carbonate to promote the uptake of molybdenum. It is interesting, that despite the positive B-Mn and negative B-Mo correlation, the B-Mn/Mo correlation is not close. Besides the effect of pH, this suggests the existence of other correlations.

The B-Na correlation of lucernes grown under operative conditions has an ecological explanation. Owing to the movement and evaporation of the water in alkali and salty soils, B and Na accumulate together, since the compounds of both elements are readily soluble. In our experimental material, the relatively high boron uptake of the halophitic taxons can also be noticed (e.g. *Limonium*).

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ESSENTIALITY OF THE TRACE ELEMENT BROMINE

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In experiments with growing, gravid and lactating goats which were repeated twice, a Br-poor nutrition (1 mg Br/kg ration dry matter) led to a significantly reduced growth, a low conception rate, reduced milk fat production, decreased haemoglobin content and a short life expectancy of the offspring intrauerinely Br-depleted. Further investigations are necessary for the clarification of the essentiality of Br.

Keywords: goats, Br-deficiency, milk quality, milk quantity.

Introduction

Together with F, Cl and I, Br belongs to the 7th group of the Periodic System of Chemical Elements. It occurs in large amounts in the biosphere: 10 to 515 mg Br/kg were found in the soil of Great Britain; on an average 54 mg/kg. Plants of this area contained between 5 and 157 mg Br/kg dry matter; on an average 45 mg/kg (Wilkins 1978). Similar Br concentrations in soils (81 and 63 mg Br/kg, resp.) and vegetation (30 and 6.4 mg Br/kg, resp.) were found in Japan (Yuita et al. 1982a, b). Compared to I, ten times more Br is taken up by the flora from the soil. Br accumulates in plants (Werchowskaja 1958).

Foodstuffs from Central Europe contain Br concentrations which exceed those of Cu and which come closer to those of Zn. Fish can particularly accumulate much Br (Grote 1980, Montag and Grote 1981). In spite of or due to its abundant occurrence, it has not yet been possible to detect significant essential functions of the trace element Br in microbes, plants, animals and humans (Nielsen 1986).

Only Oe et al. (1981) found low Br concentrations in the serum and cerebrum of patients with haemodialysis. They discovered a correlation between the insomnia observed in patients and the low Br values. Br supplementations improved the capacity of sleeping. This finding is not new. As recently as 50 years ago, Zondek and Bier (1932) reported the potential existence of a Br-containing sleeping hormone in the pituitary gland of dogs. This hypothesis has not yet been confirmed, although Br was used as a sleeping draught before barbiturates.

Within the systematic analysis of the essentiality of several trace elements (Ni, As, Li, Cd, F, Al, V), the influence of a Br-poor diet on the prenatal and postnatal growth, the reproduction performance, milk production and mortality of goats has also been investigated now.

Materials and methods

The Br deficiency experiments began in July 1986 and were repeated twice with growing, gravid and lactating goats (Table 1).

Table 1

Control and experimental goats of the Bromine Deficiency Experiment

Year	Control goats			Br deficient Goats	Remarks
	Ash	Straw	Cellulose		
1986/87	6	6	6	6	2 Br deficient goats died
1987/88	0	5	5	5	2 Br deficient goats died

Table 2

The composition of the semi-synthetic ration

Components	Amount in 100 kg
Potato starch	48.30 kg
Beet sugar	32.00 kg
Casein	10.00 kg
Urea	3.00 kg
Sunflower oil	3.00 kg
KH_2PO_4	1.34 kg
$CaCO_3$	1.00 kg
NaCl	350.0 g
$Al_2(SO_4)_3$, 60%	200.0 g
MgO	220.0 g
K_2SO_4	350.0 g
$ZnSO_4 \cdot 7\ H_2O$	50.0 g
$FeSO_4 \cdot 7\ H_2O$	45.0 g
$MnSO_4 \cdot 4\ H_2O$	40.0 g
S	35.0 g
Li_2CO_3	10.6 g
$CuSO_4 \cdot 5\ H_2O$	4.0 g
KBr	3.0 g
$Ni_2SO_4 \cdot 7\ H_2O$	3.4 g
$Cr_2(SO_4)_3 \cdot 18\ H_2O$	600 mg
$(NH_4)VO_3$	460 mg
$PbCl_2$	335 mg
NaF	220 mg
$NaWO_4 \cdot H_2O$	180 mg
KJ	100 mg
$(NH_4)_6Mo_7O_{24} \cdot {}_4H_2O$	92 mg
SeO_2	80 mg
$CoSO_4 \cdot 7\ H_2O$	80 mg
As_2O_3	40 mg
$CdCl_2 \cdot H_2O$	36 mg
Vitamin A	2000 mg
Vitamin D_3	400 mg
Vitamin E	20000 mg

Apart from the Br deficiency group, 3 control groups were included in the experiment. The cellulose group was directly compared to the Br deficiency group. Both groups only differed in the Br offer. The cellulose control goats were given about 20 mg Br/kg dry matter in their ration, the Br deficient goats < 1 mg Br/kg dry matter. Cellulose control animals took in similar Br amounts as herbivores in nature. Straw was used as litter for straw control goats and this was eaten by the animals instead of cellulose. Thus, they had an additional supply with all inorganic substances occurring in straw. In addition to the components of the synthetic control ration (Table 2), ash control animals were given 1% lignite ash; and, furthermore, they had an additional supply with all components of ash. The effect of other inorganic components of the ration, the essentiality of which has not yet been detected, was thus to be excluded.

Since the digestibility of straw is lower then that of cellulose, straw control goats gained less weight than the cellulose control animals. The ash control goats gained 11 g less weight daily than the cellulose control animals. Thus, we can proceed from the assumption that the cellulose control ration approximately meets the requirements. The goats of all groups took part in the experiment until exitus.

The composition of the semi-synthetic ration is represented in Table 2.

The animals were fed ad libitum every day. The residual foodstuff was weighed every day and the drinking water was distilled. The goats were weighed every two weeks, at 7 a.m. and their kids were weighed every 7 days.

Results

Growth

The kids of Br deficient goats had an insignificantly lower birth weight than control kids (Table 3). The difference is insignificant compared to the results obtained in other deficiency experiments with Mo, V, As, Ni, F and Cd, which were considerably greater (Anke and Groppel 1988).

After weaning on the 91st day of life, the kids were given the control and Br deficiency ration (Table 2), and their live weight gain was registered over 168 days (Table 3).

Table 3

The influence of bromine deficiency on the pre- and postnatal development of kids

Parameter (n)	Control kids		Br deficient kids		p	%
	s	$\bar{x}$	$\bar{x}$	s		
First day of life (kg) (30; 9)	0.68	2.7	2.4	0.81	>0.05	89
168 experimental days (g/day) (11; 10)	34	109	66	60	>0.05	61

Even during their intrauterine development, the kids of Br deficient goats gained 11% less weight. It was not possible to demonstrate the influence of the Br deficiency on live weight gain during the suckling period, since only 2 kids were still alive on their 91st day of life. The Br deficiency trials were continued with these 2 kids, and with 9 animals being not depleted intrauterinely. The kids with Br-poor ration gained 39% less weight gain corresponds to that of Se deficiency (Anke et al. 1987b).

Reproduction

Br-poor rations had no effect on heat intensity, but they reduced the success of the first insemination and the conception rate of goats significantly (Table 4). All control goats became gravid, whereas 43% of the animals with

Table 4

The influence of bromide deficiency on reproduction performance and life expectancy

Parameter	Control goats	Br deficient goats	P
Success of first insemination (%)	87	< 50	0.05
Conception rate (%)	100	< 57	0.01
Services per gravidity	1.1	> 1.1	0.05
Abortions (%)	0	< 12	0.05
Kids per goat carrying to terms	1.5	> 1.3	0.05

Br-poor ration did not. The number of services per gravidity was the same in both groups. This finding points to the fact that, on the average of both groups, repeated matings had the same effect on the conception rate.

In the goats which were not gravid after the first service, further matings only led to gravidity in one case. One Br deficient goat aborted. The control goats gave birth to more kids than Br deficient goats. The difference remained insignificant.

Milk and milk fat production

The Br-poor nutrition of goats reduced milk production by 20% (Table 5) The difference between control and Br deficient goats is insignificant. The

Table 5

The influence of bromide deficiency on the milk performance of goats during the first 56 days of lactation

Data	Control goats		Br deficient goats		P	%
	s	$\bar{x}$	$\bar{x}$	s		
Milk (g/day)	260	1424	1145	797	> 0.05	80
Fat (%)	0.58	3.68	3.20	0.78	> 0.05	87
Fat (g/day)	13.1	52.5	39.5	30.3	> 0.05	75

influence of Br deficiency on the fat content is less remarkable. The difference between the groups only amounts to 8%. The reduced milk production and the lower milk fat content resulted in a milk fat production of Br deficient

goats insignificantly decreased by 25%. The influence of Br deficiency on the milk fat production is greater than that found in Se, As and Cd deficient goats (Anke et al. 1987a, 1986a, b). However, the amount of produced milk would have been absolutely sufficient to meet the energy and protein requirements of Br deficient kids.

Blood picture

It was astonishing that the Br-poor nutrition of goats also influenced the haemoglobin and haematocrit values of their blood (Table 6). Compared to the control group and to the experimental F, Al, Na, Ni, As, Co, Li, Cd and V deficient goats, Br deficient goats had far the poorest haemoglobin and haematocrit level. The blood analysis presented here was carried out in January 1988, in the 3rd and 4th month of gravidity.

Table 6

The influence of bromide deficiency on the blood picture of female goats

Parameter	Control goats		Br deficient goats		p	%
	s	$\bar{x}$	$\bar{x}$	s		
Haemoglobin, mmol/l	0.89	6.8	5.7	1.0	> 0.05	84
Haematocrit, PCV	0.04	0.32	0.29	0.07	> 0.05	91
MCHC, mmol/l	0.68	21.3	19.9	1.2	< 0.01	93
Leucocytes, gp/l	2.7	8.8	9.9	3.2	> 0.05	112

As a rule, older goats had a worse haemoglobin status than younger ones (Table 7). The reduction of the haemoglobin content of the blood was stronger in 2-year-old Br deficient goats.

Table 7

The influence of age on the haemoglobin level of the blood of control and bromine deficient goats

Gravidity (n)	Control goats		Br deficient goats		p	%*
	s	$\bar{x}$	$\bar{x}$	s		
1.	0.26	7.1	6.2	1.2	0.05	87
2.	1.1	6.6	5.3	0.82	0.05	80
p	0.05		0.05			
%	93		85			

* Control goats = 100%

Mortality

The Br-poor ration of goats had no significant effect on the mortality of adult goats (Table 8), but it significantly influenced the life expectancy of

Table 8

The influence of bromine deficiency on the mortality of goats and their kids

Data	Control goats $\bar{x}$	Br deficient goats $\bar{x}$	p
Dead kids, %	0	67	< 0.001
Dead adult goats, %	18	27	< 0.05

intrauterinely Br-depleted kids. The majority of kids died during the suckling period in a good nutritional state without specific deficiency symptoms.

Discussion

Already, the essentiality of Br has been investigated repeatedly. Winnek and Smith (1937) could not register an influence of a Br-poor ration on growth, reproduction performance and health in rats over a period of 11 weeks. A small growth response to dietary trace addition of Br has been reported for chicks (Huff et al. 1956) and mice (Bosshardt et al. 1956) fed a semisynthetic diet containing iodinated casein to produce a hyperthyroid-induced growth retardation. The Br content of the basal diet was not given, and the indications of growth requirement for Br have apparently not been investigated further.

The experiments with goats fed a Br-poor ration (< 1.0 mg Br/kg ration), which were repeated twice, showed an influence on growth, conception rate, milk fat production, the haemoglobin level and the mortality of the offspring. The results so far obtained do not yet permit any statement on the essentiality of Br. They must be confirmed by at least 3 further repetitions and metabolic investigations.

Human dietary intakes of Br are large and variable. Duggan and Lipscomb (1971) obtained an average Br intake of 24 mg/day over 2 years from US diets, while Hamilton and Minski (1972/73) reported a mean intake of 8.4 mg/day from English total diets, and Varo and Koivistoinen (1980) calculated an average intake of 4.2 mg/day from Finnish diets. Actual intakes will be much higher where organic bromine compounds are used as fumigants for soils and stored grains.

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GIBBERELLIN AND SILICON ACTION UPON RICE CHLOROPLASTS

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Gibberelic acid and silicon influence the photosynthetical processes of the rice plant. GA and Si can directly change the expansion and contraction properties of rice chloroplasts. The GA influence can be neutralized with riboflavin. Si stabilised the chloroplasts' membrans while its antagonists injured the membranes (Ge) or destroyed the triphosphates (As).

Keywords: rice, *Oryza sativa* L., photosynthesis, gibberellin application, silicon application, chloroplast properties.

Introduction

Modern intensive technology of rice growing is based upon the directed usage of the biologically active matters and mineral fertilizers. Exogenous biologically active matters influence at first the system of the endogenous phytohormones, e.g. gibberellins. We have formulated the conception of the gibberellic acid (GA) action upon rice. Its principal proposition is the dissociation of the nucleotides and their protein carriers with the GA (Alyoshin et al. 1980a, Alyoshin et al. 1980b, Avakian et al. 1987). This dissociation can disturb the function of the chloroplasts' membranes (Alyoshin et al. 1980b). GA metabolic activity is connected with the rice silicon metabolism (Alyashin et al. 1980a). Si optimizes the rice plant photosynthetic processes (Alyoshin et al. 1983). Therefore, we have the basic supposition that GA and Si are able to influence directly the properties of the rice chloroplasts' membranes. The following work was the experimental verification of this supposition.

Materials and methods

Rice plants (Krasnodarsky 424 variety) were grown in the water culture until the age of two real leaves. We have shown that GA in the rice plant metabolism competes with the riboflavin (Alyoshin et al. 1980a, Alyoshin et al. 1980b, Avakyan et al. 1987), while As and Ge are the antagonists of Si (Alyoshin et al. 1980a, Alyoshin et al. 1983).In this connection we studied the plants grown in the following experimental variants: a) control — without adding of the experimental agents into the cultural solution; b) 0.001% GA into the cultural

solution; c) 0.001% riboflavin into the cultural solution; d) 0.001% GA + 0.001% riboflavin into the cultural solution; e) 60 mc/M Si into the cultural solution; f) 60 mc/m Ge into the cultural solution; g) 60 mc/M As into the cultural solution.

Chloroplasts were isolated from the plantlets leaves as described in the article (Romanko et al. 1986). The chloroplasts' expansion and contraction properties were studied as described in (Earnshaw et al. 1968).

Results

Experimental data connected with GA and riboflavin action upon rice chloroplasts' expansion and contraction are represented in Fig. 1. It can be seen that GA changes rice chloroplast properties, while riboflavin is able to

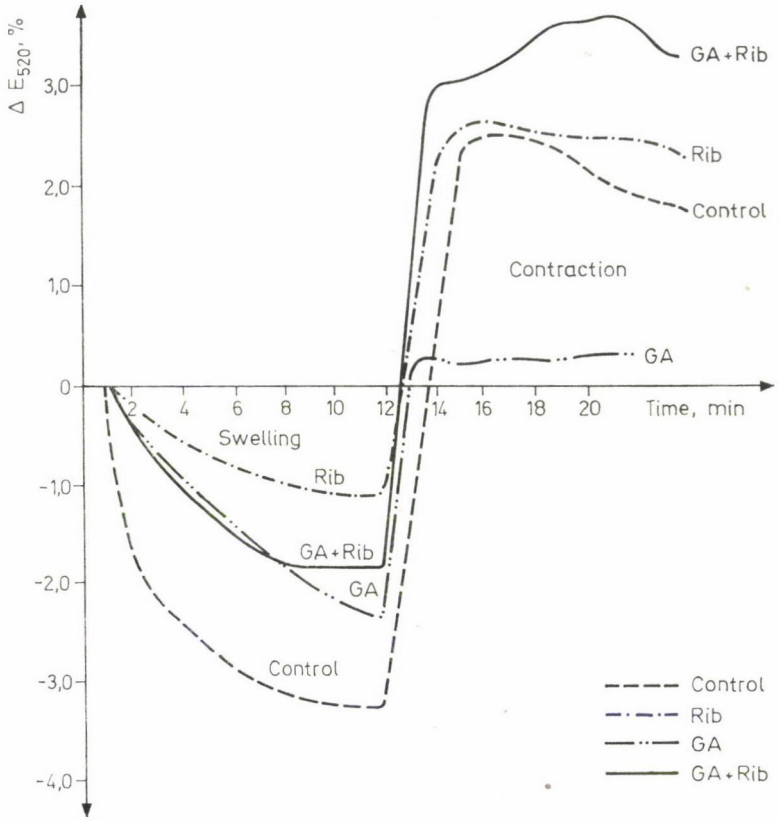

Fig. 1. Chloroplast swelling and contraction dynamics $\Delta E_{520}\%$ — the change of extinction (λ = 520 nm); $\Delta E\% = \left(\frac{E_n}{E_1} - 1\right) \times 100\%$; E_1 extinction in the first min. of measuring; E_n extinction in the n-th min. t time of measuring, min.

neutralize the activity of GA. Experimental data connected with Si and this antagonists' action upon rice chloroplast expansion and contraction are

represented in Fig. 2. All studied elements influenced significantly the rice chloroplast properties. The differences between influences of the elements are connected with the mechanisms of their activity.

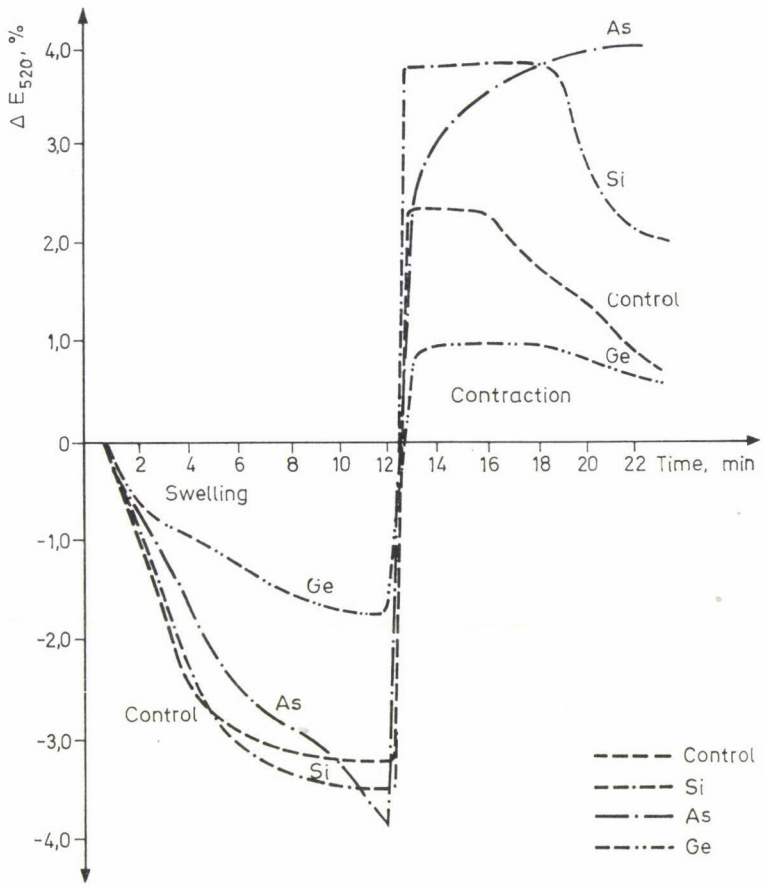

*Fig. 2.*Chloroplast swelling and contraction dynanamics.

Discussion

In our previous works (Alyoshin et al. 1980a, 1980b, Avakyan et al. 1987) we have shown that GA activity transforms the photosynthetic processes. Riboflavin can neutralize this transformation. The experimental results (Fig. 1) show that the same situation takes place in the connection of chloroplast expansion and contraction. Consequently the direct change of chloroplast properties is indeed one of the elements of the mechanism of the GA action upon rice.

Primarily, we concentrate upon the properties determined with the physico-chemical membrane characteristics (expansion, contraction). These results verify our previous suppositions based upon the investigation of some enzymes of chloroplasts (Alyoshin et al. 1980a, 1980b).

As for Si and its antagonists' activity (Fig. 2) we can say that swelling and contraction characteristics in Si-variant is very similar to the control characteristics. But Si-variant characteristics excel the control in fluctiation swing. Rice plantlets in Si-variant looked the best of all and had the best physiological characteristics (Alyashin et al. 1983). Consequently, we can conclude that Si does stabilize the chloroplasts' membranes. The plantlets of As and Ge-variants were strongly inhibited and developed the necrotic leaves. The expansion and contraction fluctuation of the As-variant chloroplasts was similar to the Si-variant one. This supports the conclusion that As does not injure the chloroplasts' membranes directly. This conclusion agrees with the published data that the main As action mechanism in the living cell is the triphosphate arsenolysis (Metzler 1980). On the contrary, Ge sharply decreases the chloroplasts' expansion and contraction fluctuation. This can be related to the direct injury of the membranes. This conclusion also agrees with the well-known experimental facts of Ge deposition in the external membranes of the microorganisms (Klapsinska et al. 1985).

As a whole, the suppositions of the GA and Si direct action upon the rice chloroplasts' membranes are varified experimentally.

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COLD STRESS RESPONSES OF INBRED MAIZE LINES WITH VARIOUS DEGREES OF COLD TOLERANCE

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The cold stress responses of two inbred maize lines. N6 and A632, which differ in the degree of early cold tolerance, were studied using various biochemical methods: determinations of proline and chlorophyll contents, and the analysis of the low-temperature fluorescence emission and excitation spectra of leaf segments. In the case of 13-day-old green plants treated at 5 °C, the proline content rose rapidly in the cold-tolerant line (A632) and slowly in the cold-sensitive line (N6). During the 6-day rewarming period the proline level dropped to the normal level in the cold-tolerant line, but remained at an above-normal level in the sensitive line, as if damage had occurred to the regenerating system of the plant. Under the effect of cold stress the chlorophyll content only decreased in the cold-sensitive line. In the low-temperature fluorescence emission and excitation spectra of 13-day-old etiolated plants, qualitative differences were found with respect to band ratios, indicating disturbances in chlorophyll biosynthesis. When the etiolated plants were illuminated, the formation of chlorophyllides could be observed in both lines, as proved by the appearance of the 690 nm emission band and the 684 nm excitation band. During the following stage of chlorophyll biosynthesis, on the other hand, differences were found between the two lines: the band shift (Shibata shift) accompanying the phytolysation of Chlide-a took place some 20 minutes later in the cold-sensitive N6 line than in the cold-tolerant A632 line.

Keywords: chlorophyll biosynthesis, cold stress, maize, prolina, Shibate shift.

Introduction

Although maize (*Zea mays* L.) is of tropical origin, it is now widely cultivated throughout in the temperate zone. Due to the differing climatic conditions, cold tolerance is an extremely important characteristic in plant production. This trait is polygenic, so a clear picture can be obtained by studying all the possible physiological responses connected with cold stress. One extremely useful physiological marker is the change in the proline content, since plants respond to various stress effects (cold, drought, high environmental salt concentration) by accumulating proline (Waldren and Teare 1974). As the result of cold or frost treatment, there is a reduction in the water uptake and transportation ability of the roots, known as physiological dryness. The increase in the proline level of the shoots cannot be attributed to the

decomposition of proteins (Pálfi and Juhász 1970), but to a rapid protective reaction aimed at preserving the osmotic value of the tissues and cells. During dry periods proline represents a reserve of nitrogen and bound water (Shiralipour and West 1984). In the course of a prolonged drought the accumulated proline is decomposed and used to maintain essential life processes until such time as the plant completely withers (Pálfi and Juhász 1970).

Various temperature effects, such as frost or abnormally high temperatures, have a strong influence on the photosynthetic process. Several research teams investigated the chloroplast-pigment content and photosynthetic activity of barley leaves at high temperatures (Dilova and Petkova 1985, Singh and Singhal 1985). The pigment content alone, however, does not provide information on the localisation and functioning of pigments within the photosynthetic apparatus. This can be conveniently studied by means of low-temperature fluorescence spectroscopy, which provides a very sensitive picture of changes in the photosynthetic apparatus under various stress conditions (Böddi et al. 1985). Further information on the method is obtained if the applied during the process of pigment formation in the cold, i.e. during the greening process of etiolated plants. During the synthesis of the chlorophylls (Chl), which play a major role in photosynthesis, protochlorophyllides (Pchlide) accumulate in plants grown in darkness. The following step in biosynthesis is the photoreduction of Pchlide to chlorophyllide-a (Chlide-a), in a light-induced reaction (Shibata 1957). However, not all the Pchlide is transformed directly. This pigment precursor contains supramolecular units, known as "forms" (Virgin 1981), which vary both spectroscopically and functionally and can be characterised on the basis of their red absorption and/or fluorescence spectra, in which the peak values are indicated as a lower or upper index to the abbreviated name of the pigment.

The main form, Pchlide $^{655}_{650}$, plays the chief role in the biosynthesis of chlorophylls (Shibata 1957, Litvin and Krasnovsky 1957). The photoreduction process takes place within a very short space of time. The transformed Pchlide $^{655}_{650}$ is regenerated by another form, Pchlide635 which is of indirect importance. This form has no fluorescence band in the fluorescence emission spectrum of etiolated leaves due to energy migration (Virgin 1981).

Another form, Pchl (ide) $^{633}_{628}$, is inactive, and is assumed to be a pigment which is not bound to protein and consists of a mixture of various Pchlide-esters (Virgin 1975, 1981). After phototransformation the Chlide $^{696}_{684}$ form appears in green plants as the result of a process involving a number of short-lived intermediate products (Sironval and Kuypers 1972, Belyaeva and Litvin 1981). In the following step, which is not light-requiring, a new form is created with a red absorption maximum at 672 nm, and a fluorescence emission maximum at 675 nm (Shibata 1957, Litvin and Krasnovsky 1957). This shift in the peak towards the blue range (blue shift) is known as Shibata shift and

usually occurs after 20—30 minutes of illumination (Virgin 1981). This shift indicates the phytolysation of Chlide-a in the biosynthesis of Chl-a.

The aim of the present work was to investigate the cold stress responses measured by changes in the chlorophyll and free proline contents in two inbred maize lines with different degrees of cold resistance. The method was tested for its applicability in plant breeding for the early indication of cold tolerance in maize lines. A further aim was to compare the chlorophyll biosynthesis processes of cold-tolerant and cold-sensitive lines by recordin gthe low-temperature emission and excitation spectra in etiolated seedlings and in those in which greening was induced by illumination.

Materials and methods

The inbred maize lines chosen for use in the experiments, the cold-sensitive (CS) line N6 (Hayes Golden) and the cold-tolerant (CT) line A632 [(B14×Mt42)×B14], showed intense differences in cold resistance on the basis of preliminary cold tests (Herczegh 1979). In these preliminary tests the plant shoots were examined at 13 °C according to Hoppe's method (1957) after a 10-day incubation at 8 °C. The cold-sensitive inbred line (CS N6) had an emergence time of 25.6 days compared with 19.8 days for the cold-tolerant line (CT A632). No significant difference in emergence rate was observed for the two lines.

In the experiments seeds originating from the previous crop were germinated at 20 °C between two strips of moist filter paper. Experiments on the line which had a longer emergence time (N6) were set up a week earlier. The germinated seedlings were planted in pots containing soil and raised for 13 days in a Conviron GB 48 climatic chamber in the phytotron of the Agricultural Research Institute of the Hungarian Academy of Sciences. During this cultivation period the day length was 16 hours, the day temperature 20 °C, the night temperature 15 °C and the relative humidity 65—75%. The light intensities applied were 30 klx, 94 W/m², 420 μmol/m²s. Three types of lamps were used as light sources: 45 pcs 40 W Krypton Superbalux incandescent lamp, 13 pcs 215 W Cool White Sylvania fluorescent lamp and 13 pcs 215 W Gro-Lux/WS Sylvania fluorescent lamp.

In the first series of experiments the plants were periodically illuminated and were exposed to a cold effect of +5 °C for 1, 2 or 3 days beginning on the night of the 13th day. Chemical analyses were carried out on leaf samples, each weighing one gram. The cold effect was followed by a 6-day normalisation, or re-warming period, during which the plants were regenerated at a constant temperature of 20 °C. In the course of the experiments changes were only made in the temperature values; all the other parameters (light intensity, relative humidity, soil moisture, nutrient supply, etc.) remained constant.

The proline content was determined using the method of Chinard (1952).

The chlorophyll content was determined as follows: The pigments were extracted with a mixture of 80% acetone and 0.01 N NH_4OH in aqueous solution. The absorption was measured at 645 and 663 nm using a Pye Unicam Sp 30 UV/Vis spectrophotometer. The equation set up by Arnon (1952) was used to calculate the concentrations.

In the second experiment, investigations were made on the greening process of etiolated plants treated at 12 °C. Six hours after the start of the cold stress, individual plants were illuminated for 5, 10, 20, 30, 35, 40, 50 or 60 minutes. Immediately after the light effect, the middle segment of the second leaf of the third-leaved plants was cut out, fixed to a glass rod and plunged straight into liquid nitrogen, where it was stored until the end of the measurements. The low-temperature (77 K) fluorescence emission and excitation spectra were revorded using a Perkin Elmer MPF 44 B spectrofluorometer. The curves were evaluated using a computer linked to the photometer. Smoothing base line correction (Böddi et al. 1985) and Gauss component fitting programmes were applied. Since only very slight differences in chlorophyll content could be observed during the first hour of greening, spectral changes caused by self-absorption did not hinder the comparison of the fluorescence spectra.

The chemical and spectroscopic analyses were carried out in three replicates on three parallel samples.

Results and discussion

In the first series of experiments, studies were made on changes in the chlorophyll and free proline contents of plants raised under normal conditions and the cold-treated. The values obtained can be seen in Figures 1 and 2.

Cold treatment was carried out for 1, 2 or 3 days and the resulting changes were measured. As the result of the cold effect the free proline content changed in both lines (Fig. 1). In the cold-tolerant line the concentration rose sharply, while in the cold-sensitive line it rose slowly to a similar level. Thus, within a single species differences can be found between lines as a result of

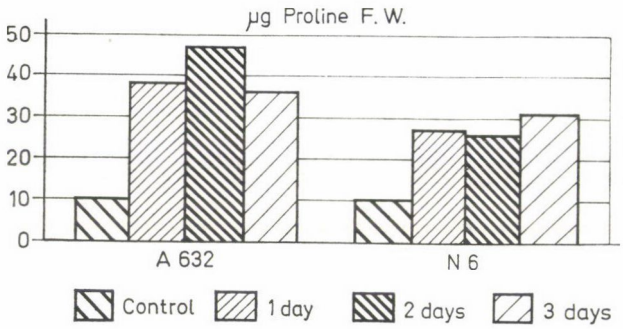

Fig. 1. Changes in the free proline content in cold-treated leaves of maize lines CT A632 and CS N6

cold stress. Stewart and Voetberg (1987) observed a similar phenomenon when studying the drought tolerance of wild and wilting mutant tomato plants, where the wilting mutant accumulated less proline, but a more rapid rate, while the wild type accumulated more proline, but more slowly, during the wilting treatment lasting for a day and a half.

In freezing studies, Stefl and co-workers (1978) measured the proline accumulation in the shots of winter rape and winter wheat. They found a measurable increase in the free proline content after 72 hours of freezing, while in the present experiments the effect of cold became visible after 24 hours. It can be assumed that this difference is due to the differing cold tolerance of the experimental material.

The proline values measured after the 6-day regeneration period were also different for the two lines (Figure 2). In the CT A632 line the proline level dropped proportionately to the normal level. In the CS N6 line, after the longest (3-day) cold treatment, the proline level did not drop to the initial concentration, but was significantly higher in all phases of the experiments than that of the control, as if the regeneration system of the plant had been damaged by the cold.

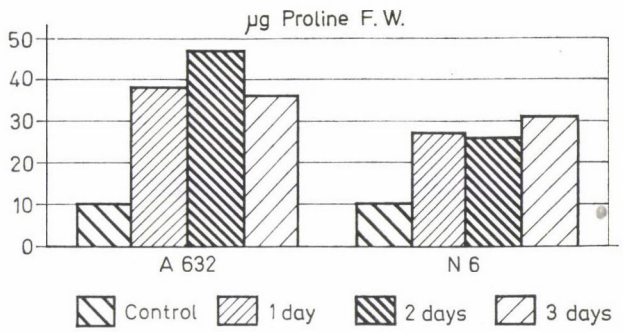

Fig. 2. Changes in the free proline content in cold-treated leaves of maize lines CT A632 and CS N6 subsequently regenerated for 6 days at 25 °C

The fact that the free proline content was perceptibly higher in cold-treated plants, and that the accumulation curves showed differences between the two types, means that the method can be applied as a marker in plant production and breeding. This result agrees with those obtained by Waldren and Teare (1974) and Hanson and co-workers (1977).

Changes in the chlorophyll content as the result of cold treatment differed in the two lines (Figure 3). In the cold-sensitive line (N6) there was a more intensive drop in the Chl content compared to the control, while in the tolerant line this drop was not significant. If the plant was exposed to cold stress in its green state, the photosynthetic apparatus only suffered damage after a lengthy period of cold, operating apparently normally for a considerable time. This was experimentally proved by Taylor and co-workers (1987), who investigated the activities of various enzymes playing an important role in the photosynthesis in illuminated millet and maize plants at

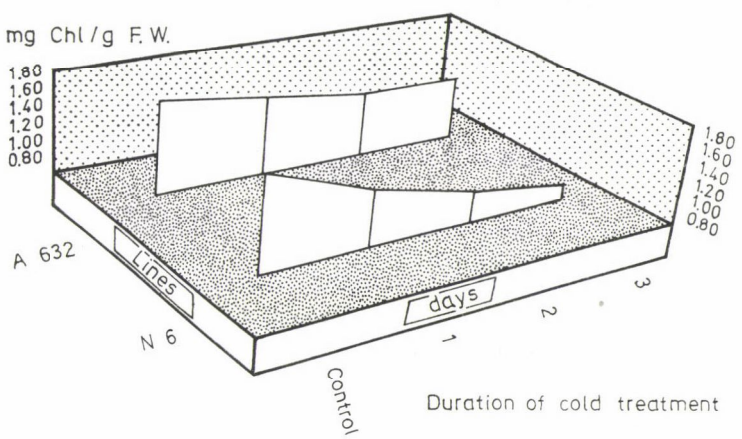

Fig. 3. Changes in the total chlorophyll content in cold-treated leaves of maize lines CT A632 and CS N6

a temperature of 10 °C. With two exceptions, the activity of all the enzymes was found to be normal, and the activity of the enzyme catalase only dropped in maize if the cold effect was 6 °C and there was an increase in light induction.

Wise and Naylor (1987) studied changes in the concentrations of photosynthetic pigments in cucumber leaf segments, as a result of freezing under illuminated conditions. In the frost-sensitive leaves of cucumber there was a decrease in the quantities of Chl-a, Chl-b, β-carotene and three xanthophylls in the cold, while in the leaves of peas concentrations did not change. This led to the conclusion that the decomposition of these pigments is caused by photooxidation and thus by lipid peroxidation. Tolerant plants, however, are able to compensate for the damaging effects of photooxidation (Wise and Naylor 1987). In the current experiments the reduction in total chlorophyll content in the CS N6 line may well be due to photooxidation, but this must be confirmed by further chemical analyses; for instance, by measurements on cold-induced changes in the concentrations and lipid compositions of other photosynthetic pigments.

During the 6-day regeneration period the Chl content in the tolerant line was almost identical to that in the untreated sample, and the Chl level in the cold-sensitive line also returned to normal (Figure 4). The only exception

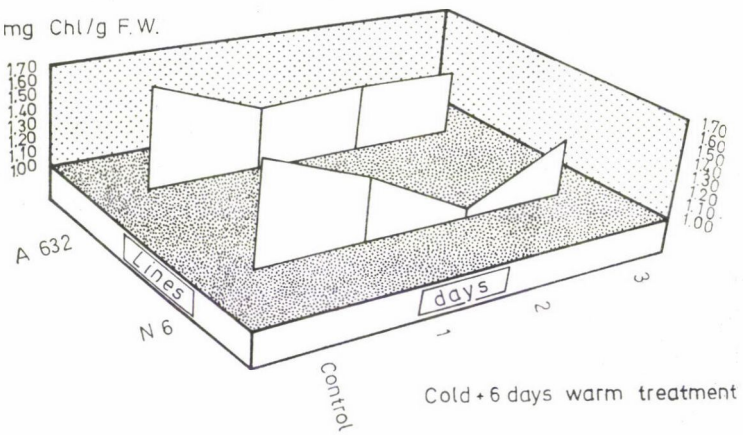

Fig. 4. Changes in the total chlorophyll content in cold-treated leaves of maize lines CT A632 and CS N6 subsequently regenerated for 6 days at 25 °C

was the 2-day cold treatment, in the course of which the Chl concentration remained at a low level. A similar anomaly was observed in the Chl-a/Chl-b ratio (Figure 5).

Since the quantitative changes in the Chl contents of green plants did not prove to be a satisfactory indicator for the characterisation of physiological processes induced by the cold effect, examinations were made to deter-

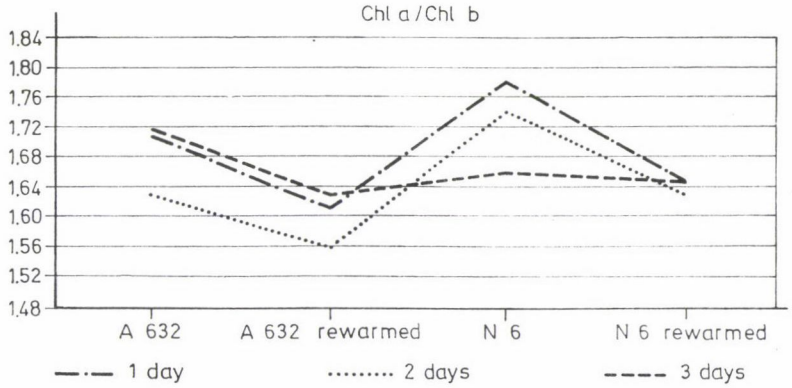

Fig. 5. Changes in the Chl-a/Chl-b ratio during 1, 2 or 3 days of cold treatment, and after a 6-day regeneration period following cold treatment

mine whether there was any differences in the chlorophyll biosynthesis of the two maize lines. The low-temperature fluorescence and excitation spectra of the treated leaves were used for this purpose. The spectra of etiolated, cold-treated leaves are shown in Figure 6 and the curves recorded after illumination in Figure 7.

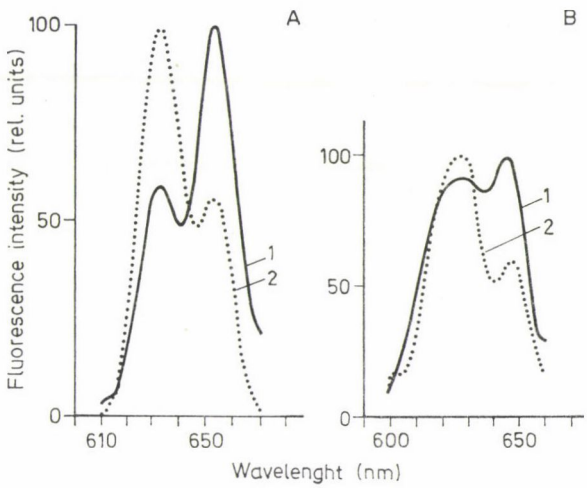

Fig. 6. Spectra recorded for leaf segments of CT A632 (Curve 1) and CS N6 (Curve 2) at 77 K after cold stress (6 h at 12 °C) and prior to illumination. *A:* Fluorescence emission spectra (excitation wavelength: 440 nm), *B:* excitation spectra (emission recorded at 750 nm)

In the etiolated leaves of both lines, the maxima of the emission and excitation spectra were situated in the same position (Figure 6). The course of the curve recorded for the CT A632 line was found to be identical to that

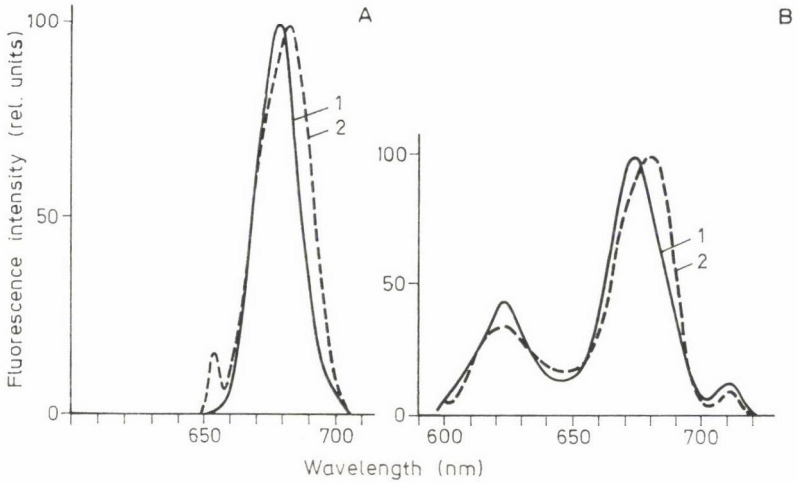

Fig. 7. Spectra recorded for leaf segments of CT A632 (Curve 1) and CS N6 (Curve 2) at 77 K after cold stress (7 h at 12 °C) and illumination for 60 min. *A:* Fluorescence emission spectra (excitation wavelength: 440 nm), *B:* excitation spectra (emission recorded at 750 nm)

observed for other angiosperm leaves, but the picture presented by the CS N6 curve was altered by cold treatment. A change was observed in the relative quantities of the components. There was a considerable increase in the quantity of the component with an emission maximum at 633 nm and a reduction in that with a maximum at 655 nm. Similar changes were seen in the excitation spectra (Figure 6/B). According to Virgin (1975) the rise in the 633 nm emission maximum and the 630—632 nm excitation maximum indicates the presence of a large quantity of Pchlide or Pchl in an inactive form which is unable to take part in Chl biosynthesis. Certain Pchlide forms remain active, however, and these enable the biosynthesis of Chl to continue. In order to trace the Chl biosynthesis process further, etiolated plants were illuminated for increasing lengths of time and the spectra of the treated plants were recorded, as seen in Figure 7.

In both maize lines the 696 nm emission maximum and the 684 nm excitation maximum characteristic of Chlide-a appeared in the spectrum under the effect of light, since photochemical reduction is not influenced by the cold treatment (Sironval and Brouers 1970). A substantial difference was found, however, in the chlorophyll biosynthesis step. In the spectra of tolerant plants the maxima shifted towards the blue range (Figure 8). After 30 minutes of illumination the emission band appeared at 675—680 nm and excitation peak at 672 nm.

In the experiments, maize lines with differing degrees of cold tolerance proved to have very useful biochemical markers, a knowledge of which could

well be important in the production and characterisation of new varieties and hybrids.

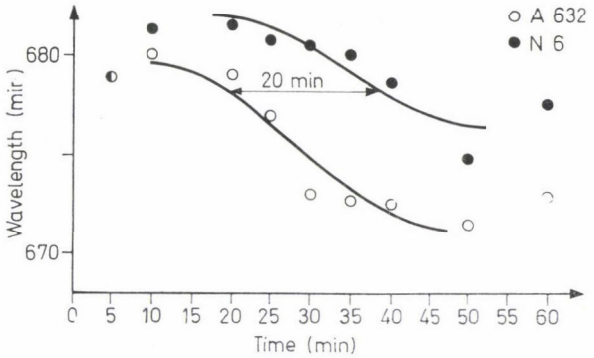

Fig. 8. Migration of the peak observed at 670—68 nm in the excitation curves of lines CT A532 and CS N6 after illumination under cold stress

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SUBSTITUTION ANALYSIS OF FROST RESISTANCE IN WHEAT IN *IN VITRO* SOMATIC CULTURES AND AT SEEDLING LEVEL

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The substitution series of Chinese Spring/Cheyenne was examined for frost resistance at seedling stage and in callus cultures induced from immature embryo and young leaves under the coleoptile. The survival of the callus was determined by TTC treatment following hardening and freezing at −12 °C. The callus level examination of the substitution lines suggests that frost resistance demonstrable at the plant level can be analysed by *in vitro* methods as well. The difference between recipient and donor variety can be unambiguously pointed out in callus cultures, and so can the positive effect of the chromosomes *5A* and *5D* on frost resistance. The effect of the chromosomes *6A* and *3D* has not been observed at plant level so far, though in *vitro* cultures they have proved remarkably effective. This calls attention to the effect of plant organization on frost resistance. The origin of the explant did not influence the development of the property. Plant regeneration after freezing offers a possibility for elaborating in vitro mutant selection techniques.

Keywords: *Triticum aestivum* L., chromosome substitutions, callus culture, frost resistance, effect of explants.

Introduction

The genetic control of frost resistance in winter wheat has been studied by many authors under both artificial and natural conditions (see for review Veisz et al. 1987). The results of the *F2* monosome analyses and of the chromosome substitution analyses suggest that some chromosomes are responsible for the development of frost resistance (Goujon et al. 1968, Lew and Jenkins 1970, Jenkins 1971, Sutka and Rajki 1978, Sutka 1981, Musich and Bondar 1981, Sutka et al. 1986), and that the property is under a highly complex polygenic control (Cahalan and Law 1979, Puchkov and Zhirov 1978, Sutka et al. 1986). Further, the data unequivocally suggest that, out of the chromosomes determining the character, those of the homologous groups 5 carry the most effective genes responsible for frost resistance (Cahalan and Law 1979, Poysa 1984, Sutka et al. 1986). According to the result of the analysis of frost resistance in the Chinese Spring/Cheyenne substitution series among the chromosomes of the homologous group 5, the *5A* chromosome may be the most important in respect to this property (Sutka 1981, Poysa 1984, Sutka et al. 1986). An examination of the *5A* chromosomes of the different

varieties reveals a considerable allelic polymorphism; the various *5A* chromosomes may produce different effects from high frost resistance to definite frost sensitivity (Sutka and Kovács 1985).

On the basis of literary data and our own investigations we may say that at plant level we have achieved a comprehensive view of the chromosomal control of frost resistance. Today the range of the chromosome donor varieties, with the help of which the forst resistance of the varieties grown in Hungary can be improved, is relatively easy to determine. However, if the development and functioning of the character are to be better understood, further investigations are required. The complexity of the development and functioning of the character, its polygenic nature, the high environment dependence can be explored only by many-sided parallel examinations. It seems reasonable, therefore, that examinations at seedling level are completed with *in vitro* analyses. The *in vitro* analyses may supply highly important information on the manifestation of the character at cell- and tissue level, and on the influence of the plant organization on frost resistance.

Frost resistance among *in vitro* somatic cultures was first studied by Duda and Kacperska (1833) who used *Populus nigra* L. and *Brassica napus* L. callus cultures for their experiments. Since then some have also dealt with the question in the case of wheat (Chen and Gusta 1986, Galiba and Sutka 1988). These data and the results of investigations carried on at our institute unequivocally prove that frost resistance can be tested well at callus level too, and the effect of the most important chromosomes is demonstrable (Galiba and Sutka 1988, Kovács 1988).

In our present paper we try to answer the question of how the origin of the explant influences the manifestation of frost resistance at callus level, and whether the various organization levels have any role in the expression of the character.

Materials and methods

Plant material

In our experiments the full Chinese Spring/Cheyenne substitution series as well as the donor- (Cheyenne) and the recipient (Chinese Spring) variety were studied. The substitution series was produced by R. Morris at the University of Nebraska, USA.

In order to attain the highest possible genetic homogeneity of the experimental material, the substitution series and the two varieties were maintained with the single seed descent *(SSD)* method for three years, then the seeds obtained in the last year of the cycle were sown in autumn at optimum time in medium compact forest soil worked like a graden at a spacing of 20×20 cm. During the vegetation season of the following year we marked out those plants of the overwintered stand which produced at least 10 fertile spikes, a sufficient number for the purpose of examinations. With 150 immature embryos collected from the plants marked out in each of the 21 lines and the two varieties, callus cultures were induced, and the remaining grains were harvested after ripening. From a part of the grains a plant control group was formed which was then examined together with the callus cultures of immature embryo origin. The remaining grains were divided in two parts. One half was ger-

minated; then, from the young leaves under the coleoptile, callus was induced again in order to study the effect of explants of different origin; the other half of the grains was similarly used to form a plant control.

Callus induction

The callus cultures were induced from two different explants: immature embryos and young leaves under the coleoptile. In the first case the time of flowering was surveyed in the field, and the calluses were induced from 12 to 14-day-old immature embryos after sterilization usual in tissue cultures. In the second case the ripe grains gathered in were sterilized and cold treated (at 4 °C for 24 hours), then germinated under sterile conditions. The cultures were induced from young leaves under coleoptile. According to our preliminary studies the stage most suitable for callus induction is when the coleoptile is 1—2 cm long.

The callus induction took place in both cases on Murashige—Skoog (1962) culture medium modified by Sears and Deckard (1982) at 50 mg/l adenine and 2 mg/l 2,4-D concentration.

In the first four weeks the callus cultures were kept in the dark at 26 °C. After four weeks the material was passed over to the same culture medium and kept for further four weeks at 26 °C with 16 hours of illumination (240 μE/s/m).

Hardening and freezing

After the eighth week 100 calluses in both cases were passaged (to the same culture medium), then covered with aluminium foil and together with the plant control (100 plants per line and variety) hardened by the method of Sutka (1984). At the end of the six-week hardening period the material in both experiments was frozen at —12 °C.

After freezing the plant control was evaluated by the method of Sutka (1981). For 24 hours after freezing the calluses were kept at 26 °C, then tested for viability with a simplified form of the triphenyl-tetrazolium-chloride (TTC) method (Steponkus and Lauphear 1967). The composition of the TTC solution was: 0.1 mg/l BA, 0.5 mg/l NAA, 2 mg/l 2,4-D, 0.5 mg/l nicotinic acid, 0.5 mg/l B_6 vitamin and 0.08% TTC. Each Petri dish was filled with 10 ml solution, and 18 hours later the surviving calluses were counted (survival percentage) on the one hand, and the survival was evaluated on the basis of staining by points from 0 (unstained) to 5 (stained to dark red), on the other hand. For the evaluation the staining of the control (unfrozen) calluses was used on the assumption that differences in staining may also have been among the lines.

The experiment data were evaluated by variance analysis (Sváb 1981). The percentage values were transformed into arcsin $\sqrt{x}$ form in order to avoid the great heterogeneity of the variance (Johnson 1963).

Results

From both the young leaves under the coleoptile and the immature embryos, callus was induced practically in 100% on the culture medium used. The frequency of callus induction was influenced neither by the origin of the substitution lines nor the explants, neither was there any difference in the time of induction.

The viability test with TTC proved highly efficient in analysing the viability of the calluses after freezing. The calluses necrotized in the course of freezing remained totally white, while the surviving ones stained different colours depending on their respective frost resistances. The most viable calluses were dark red. Only those calluses were considered lost which did not show staining at all. However, it happened in many cases that a part of a callus with poor frost resistance stained bright red, while more than 95%

of it remained white, i.e. dead. These stained parts were at the same time centres of regeneration showing intensive division of cells.

In Table 1 the survival data of calluses of immature embryo origin are shown together with the data of the plant control. On the basis of the survival

Table 1

Trend of forst resistance in calluses and seedlings induced from immature embryos of the Chinese Spring/Cheyenne substitution series

Varieties and lines	Survival %		Evaluation by points	
	plant	callus	plant	callus
Chinese Spring (st)	37.8	52.5	1.13	1.73
Cheyenne	97.8***	91.3***	3.88***	3.98***
1A	33.3	51.3	0.99	0.95
2A	22.2	47.5	0.79	1.23
3A	20.0	58.7	0.74	1.90
4A	35.6	55.0	1.19	1.43
5A	74.4***	85.0***	2.29***	3.40***
6A	32.2	85.0**	1.09	3.50***
7A	51.7*	85.0**	1.67*	3.03**
1B	37.8	21.3	1.14	0.53
2B	37.8	45.0	1.18	1.20
3B	34.4	52.5	1.24	1.75
4B	57.8*	67.5	1.67*	2.03
5B	50.0	37.5	1.56*	1.45
6B	33.0	41.3	1.14	1.85
7B	40.0	72.5	1.19	2.48
1D	43.3	53.8	1.26	1.53
2D	25.6	37.5	0.92	1.28
3D	38.9	78.8*	1.27	2.88**
4D	28.9	50.0	1.02	1.78
5D	76.7***	76.3*	2.37**	3.08**
6D	33.9	68.8	1.09	2.20
7D	40.0	71.3	1.23	1.90

*, **, *** — at P = 0.05, 0.01 and 0.001 it differs from Chinese Spring
st — variety (recipient) representing the basis of comparison

percentage there is an essential reliable difference between the two varieties both at plant-and at callus level, the Cheyenne proved frost resistant in both cases. Among the substitution lines the *5A*, *7A*, *4B* and *5D* chromosomes improved reliably the frost resistance of the Chinese Spring in the case of seedlings. At callus level the picture was somewhat different; here the *5A*, *6A*, *7A*, *3D* and *5D* chromosomes increased significantly the frost resistance of the recipient variety.

The differences of the substitution lines are better characterized by the result of scoring than by the survival percentage. On this basis the chromosomes *5A*, *7A*, *4B*, *5B* and *5D* significantly increase the frost resistance. The

frost resistance of the recipient variety Chinese Spring was improved in the greatest measure by the *5A* chromosome. On the other hand, in the case of several chromosomes there is a difference in frost resistance between the calluses and the plants. At callus level the examination of seedlings gave reliable results for the effect of the *5A*, *7A* and *5D* chromosomes. Besides them the effect of the *6A* and *3D* chromosomes also proved critical at callus level, while the chromosomes *4B* and *5B* were not found here to have a positive effect on frost resistance.

In Table 2 the survival after freezing of calluses induced from young leaves under the coleoptile is shown. The trend is similar to the above. On the

Table 2

Frost resistance of calli induced from young leaves under coleoptile of the Chinese Spring/Cheyenne substitution series

Varieties and lines	Survival %		Evaluation by points	
	plant	callus	plant	callus
Chinese Spring (st)	35.6	52.5	1.07	1.43
Cheyenne	97.8***	97.5***	3.82***	4.18***
1A	17.8	52.5	0.60	1.53
2A	20.0	60.0	0.73	1.35
3A	22.2	50.0	0.80	1.63
4A	26.7	65.0	0.96	1.65
5A	64.4**	87.5**	2.73***	3.28***
6A	31.1	77.5*	1.13	3.08***
7A	45.6	95.0**	1.68*	2.67**
1B	31.1	20.0	0.98	0.92
2B	48.9	50.0	1.58	1.38
3B	35.6	47.5	0.80	1.78
4B	53.3*	70.0	1.70*	2.23
5B	46.7	37.5	1.71*	1.08
6B	17.8	27.5	0.79	1.53
7B	37.8	72.5	1.20	2.07
1D	35.6	55.0	1.13	1.55
2D	22.2	27.5	0.91	1.12
3D	24.4	84.5**	0.91	2.72***
4D	22.2	50.0	0.69	1.65
5D	75.6***	77.5*	2.47***	2.85**
6D	24.4	67.5	0.82	2.52
7D	44.4	67.5	1.38	2.27

*, **, *** — at P = 0.05, 0.01, 0 001 level it differs from the recipient Chinese Spring
st — standard variety.

basis of the survival percentage, at seedling level the chromosomes *5A*, *4B* and *5D* (the effect of *7A* could not be determined here) reliably increased the frost resistance of the Chinese Spring, while at callus level the effect of the

5A, *6A*, *7A*, *3D* and *5D* chromosomes was decisive. When evaluating the results of scoring at plant level the chromosomes *5A*, *7A*, *4B*, *5B* and *5D* deserve mentioning. Here again the effect of the *7A* chromosome can be observed. The results of the examination of calluses were identical with the data obtained for the calluses of immature embryo origin, with the exception of the chromosome *6D*, the effect of which was not noticed there.

Conclusions

According to the data of literature for the inheritance of frost resistance the *5A*, *7A*, *2B*, *4B*, *5B*, *5D* and *6D* chromosomes are primarily responsible (sutka 1981, Puchkov and Zhirov 1978, Sutka et al. 1986). Out of them the chromosomes of the homologous group 5. carry the genes of the greatest (influence (Poysa 1984, Sutka and Kovács 1985, Sutka et al. 1986). Not every critical chromosome is able to ensure the survival of the lines at the different freezing temperatures. According to our earlier investigations, it was only with the *5A*, *7A* and *5D* chromosomes that their effect could be demonstrated in every case after freezing at −12 °C. The results obtained in the present case are practically the same, with the sole exception that the effect of the *4B* and *5B* chromosomes occasionally could also be detected in the experiments. This, too, supports those earlier results which emphasize the importance of the homologous group 5.

The callus level examinations show that frost resistance demonstrable at plant level can be analysed by *in vitro* methods as well. Results of earlier experiments prove that the process of hardening takes place in the case of both individual cells and calli (Steponkus 1972, Tumanov et al. 1977, Chen and Gusta 1983), so the frost resistance of varieties can be tested in *in vitro* somatic callus cultures (Chen and Gusta 1986, Kovács 1988). Galiba and Sutka (1988) studying some chromosomes of the Chinese Spring/Cheyenne substitution series found that the favourable effect of the *5A* chromosome on frost resistance could also be detected in somatic callus culture. Unfortunately they did not give results of the full substitution series. Earlier data suggest that frost resistance can be relatively quickly and simply analysed in *in vitro* somatic callus cultures.

However, our results obtained in analysing the full substitution series do not quite show the same.

The significant effect of the *5A* and *5D* chromosomes can be unambiguously demonstrated in callus cultures. In the course of analysing the survival of either the plant material or the calluses, these two chromosomes were found to improve the frost resistance of the recipient variety Chinese Spring to a considerable extent. The chromosomes *7A* and *6D*, which in the

earlier frost resistance tests several times showed a critical effect, have the stronger influence at callus level than at plant level, while the effect of the *4B* and *5B* could not be observed at callus level. At callus level, two chromosomes *(6A* and *3D)*, which had not so far shown any positive influence on frost resistance, were now found to increase it essentially. This suggests that plant organization, both the individual and tissue organization, may substantially modify the frost resistance developing at cellular level. The ineffectiveness of the *2B* and *4B* chromosomes is probably due to the absence of photosynthetic sugar accumulation, because the effect of the chromosomes in freezing tests was greatly influenced by the time and intensity of illumination (Veisz 1988). On the effect exercised by the *6A* and *3D* chromosomes on frost resistance, we have not so far determined anything. The fact that we hardened the calluses in darkness thus excluding the effect of light on frost resistance, unambiguously suggests that hardening is induced by the low temperature, and the process of vernalization takes place at callus level exactly as in the case of whole plants.

The different origin explants practically influence neither the process of vernalization nor the frost resistance of calluses of the substitution lines. This suggests that any plant part from which callus culture is successfully induced can probably serve as basic material in *in vitro* examinations of frost resistance.

On the basis of our results we may assume that the use of *in vitro* somatic callus cultures will essentially facilitate the biochemical and genetic studies of frost resistance, while parallel examinations at plant- and callus level may disclose the effects of individual organization.

The examination of calluses of frost sensitive lines offers a wider possibility. The fact that with the TTC method regeneration centres showing intensive cell division are primarily stained, while the rest of the callus does not stain, renders possible the quick identification of cell groups surviving the stress conditions. On this basis the examination of various chromosomes for phenotypic stability, and the chromosome dependent estimation of the number of stress specific mutations, may become possible. Namely, the cell groups which, as in the present case, survived freezing are supposed to be such mutant regions whose frost resistance is essentially better, compared to the rest of the callus. With all this taken into consideration, the possibility exists of both elaborating mutant selection methods and estimating the stress specific mutation rate. Despite this, we may expect that cell groups showing stress resistance at callus level will not necessarily possess the same degree of resistance, as is suggested by the organization differences. Even if the regenerated plant carries the changed phenotype we cannot be sure that this character will be steadily transmitted. Further intensive research work is required to answer these questions.

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EFFECTS OF GIBBERELLIC ACID (GA_3) ON CYTOPLASMIC MALE STERILES AND THEIR MAINTAINER/RESTORER LINES IN RICE

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The effects of gibberellic acid (GA_3) on the fertility of two cytoplasmic male steriles carrying WA cytoplasm, and on their corresponding maintainer/restorer lines were studied in rice. While GA_3 failed to restore the fertility of cytoplasmic male steriles, its effects on maintainer/restorer lines indicated that it can effectively be used to shift the normal plants toward femaleness.

Keywords: rice, Oryza sativa L. 3, cytoplasmic male steriles, gibberellic acid.

Introduction

More than 95% of the area under hybrid rice cultivation in China is devoted to hybrids derived from a single source of cytosterility, designated as S_2 (earlier designated WA), because other cytosterility systems are not as stable or lack effective restorers. This situation makes F_1 rice hybrids potentially more vulnerable to diseases and insect pests that could be associated with cytoplasmic factor. In recent years, as an alternative to the cytoplasmic — genetic system, increasing emphasis has been given to the possibility of chemically suppressing and/or restoring the fertility of the genotypes to facilitate their use in the production of commercial F_1 hybrids (Greyson and Walden 1976, Hockett et al. 1976, Sneep et al. 1979). However, as compared to the possibility of using chemicals to revert nuclear-based male steriles to produce seeds, far less attention appears to have been directed to the chemical restoration of cytoplasmic male sterility. While the use of an effective chemical pollen suppressant would obviate the need for genetic or cytoplasmic-genetic male sterility, the chemical restorer(s), if available, could effectively be used to replace the maintainers *(B lines)* in the hybrid production system.

Gibberellic acid (GA_3) has been shown to induce either partial or almost complete male sterility in several crops, including onions (Van Der Meer and Van Bennekom 1973, Van Bennekom and Van Der Meer 1978), corn (Hansen

et al. 1976), sunflower (Köves et al. 1978), lettuce (Eenink and Vereijken 1978) and wheat (Smart and Marshall 1985). Although GA_3 has also been reported to restore the fertility of genetic male steriles in corn (Nickerson 1960), barley (Kasembe 1967) and tomatoes (Sawhney and Greyson 1973), yet the specific information with regard to its role in reinforcing or mitigating the sterilizing effect, and/or restoring the fertility of S_2 cytosterility system, is lacking. The present study records the effects of gibberellic acid on the fertility of cytoplasmic male steriles and their corresponding maintainer/restorer lines in rice.

Materials and methods

The materials of the present study comprised two cytosterile lines of rice (*A lines* — V 20 A Zhen Shan 97 A) carrying WA cytoplasm, their respective maintainers (*B lines* — V 20 B and Zhen Shan 97 B) and common restorer (*R line* — PC 19). Twenty seedlings of each *A* (cytosterile), *B* (maintainer) and *R* (restorer) line were transplanted in the field with a spacing of 40×20 cm and standard agronomic practices were followed. Ten plants of each line were sprayed weekly with 300 ppm gibberellic acid from one month after transplanting until flowering was completed. The remaining untreated 10 plants served as controls. The seed set/panicle was used to measure the effective male fertility.

Results and discussion

The data on seed set/panicle clearly indicate that GA_3 does not restore the fertility of cytoplasmic male sterile lines carrying WA cytoplasm (Table 1). In fact, GA_3 appeared to reinforce rather than mitigate the effect of WA cytoplasm. The results are conform with the previous findings noted earlie.

Table 1

Mean and range in seed set/panicle for control and GA_3 treated A(sterile), B(maintainer) and R(restorer) lines of two rice genotypes

Genotype	Line	Control		GA_3 Treated	
		Mean	Range	Mean	Range
V 20	A	4.9	0—12	0—5	0—1
	B	74.4	52—88	1.1**	0—5
Zhen Shan 97	A	6.2	2—14	0.	0
	B	75.7	51—101	6.2**	0—27
PC 19	R	89.7	48—135	15.5**	4—31

** Significant at 1% level.

in corn, onion, sunflower, lettuce and wheat. According to Sawhney and Greyson (1973) added gibberellins induce maleness in systems where there is an inhibition or abnormality in stamen development, whereas they promote

femaleness in systems with normal stamen development. The failure of GA_3 to promote maleness in *A lines* suggests that sterilizing effect of WA cytoplasm is possibly due to increased gibberellin synthesis or increased GA_3 sensitivity at some critical stage(s) in pollen development. If this explanation is valid, it is probable that the use of antigibberellins such as cytokinins or abscissic acid (Rudich and Halevy 1974, Ahokas 1982) may prove effective in fertility restoration. On the other hand, the effects of GA_3 in significantly reducing the seed fertility in B and R lines suggest that gibberellins also play a very significant role in regulating sex expression in rice as they do in other species. GA_3 induced male sterility in *B* and *R lines* apparently supports the latter part of Sawhney and Greyson's statement and is indicative of an increase in auxin content or an imbalance of relative amounts of GA_3 or other related plant hormones (Smart and Marshall 1985). While GA_3 failed to restore the fertility of cytoplasmic male sterile lines, its effects on *B* and *R lines* clearly indicate that it can effectively be used to shift normal plants toward femaleness.

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EFFECT OF GAMMA RADIATION AND TEMPERATURE ON POTATOES DURING STORAGE

I. CHANGES IN TEXTURE AND WEIGHT LOSSES

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Radiation treatment of potato was carried out by gamma irradiation from a Cobalt-60 source. The radiation doses applied for two varieties of potato (Desire and Metal) were 50 Gy, 100 Gy and 500 Gy. Irradiated and control potatoes were stored at 5 °C and 20—25 °C for 6 months. Texture investigation, sprout growth rate, and weight loss were measured every month. At the end of storage the control samples were soft and shrunken, but the texture of irradiated samples stored at 5 °C was much better. Sprout growth was completely inhibited in potatoes which had received dosages of 100 Gy and 500 Gy. Weight loss increased during storage time, and decreased by the irradiation treatment, depending on the variety and temperature. Higher weight loss due to rotting was observed in the irradiated tubers than in the control ones. In the case of Metal variety, higher values of weight losses due to respiration, evaporation and rotting were observed in the irradiated tubers than in the control ones.

Keywords: gamma radiation, potatoes, storage.

Introduction

The use of ionizing radiation as a food preservation technique is relatively new. It is a physical method, so the contamination by chemicals is eliminated. Food preservation by radiation is also effective for energy saving during storage, for control of spoilage organisms in perishable foods and for inhibition of physiological processes such as sprouting in tubers and ripening in fruits. Irradiated tubers appear more attractive than the control ones and due to their more favourable texture they are also more suitable for processing. Products treated by irradiation have improved colour, flavour and texture (Sreenivasan, 1974). The optimum dose of radiation used for potato is 125 Gy which is better in change of shrivelling, texture and extended the storage period by more than 4 months, compared with unirradiated ones at a natural low temperature in a storage room (Cho et al., 1982). The softening of potatoes is a function of storage time. The control tubers softened significantly ($P = 0.1—5\%$) more than that of treated tubers (Beczner, 1983). It is known that the ionizing radiation is used in the inhibition of germination for potato, onion, carrot and sugar beet. The main dose reported by several investigators,

which causes an irreversible sprout inhibition, is 100—140 Gy (Jaarma, 1968).

Some of the authors found little difference in spoilage between radiation-treated and control tubers, while others observed more extensive spoilage in irradiated potatoes than in the control ones (El-Sayed, 1977, Beczner, 1980, Hayashi and Kawashima, 1982).

Material and methods

Two types of potato varieties were used in these investigations; Desire and Metal. The potatoes were grown in the state farm of Szentlőrinc (in the south of Hungary). Radiation treatment of potato was carried out by gamma irradiation from Cobalt-60 source at the pilot plant of AGROSTER Irradiation Company, Budapest. The radiation doses applied for both varieties were 50 Gy, 100 Gy and 500 Gy. Irradiated and control potatoes were collected in cases of 20 kg per treatment and stored at 5 °C, also the same amount from each treatment for both varieties was stored at 20—25 °C. Texture, sprouting and weight loss were measured regularly every month from December 1987 to June 1988.

Texture investigation

Texture evaluation measurements were carried out with potatoes of different treatments, using testing machine 250 kp. Type FM 250. A cylinder of 29 mm in diameter was cut from 3 potatoes of each treatment of both varieties. Height varied from sample to sample. Each sample was placed on the flat plate of the instrument and the moving horizontal head pressed the sample until the breaking point. Force was measured as the height of the peak of the force deformation curve (measured diagram). The reading is in kg, and was taken from the instrumental scale (F). The surface area (A) of samples, stress (σ) and specific deformation (ε) were calculated as follows:

$$A = \frac{d^2\pi}{4} \text{ mm}^2; \qquad \sigma = \frac{F}{A} \text{ kg/mm}^2; \qquad \varepsilon = \frac{\Delta\lambda}{H}$$

where

H = sample height
$\Delta\lambda$ = deformation
Young modul as (E) of each sample was calculated

$$E = \frac{\sigma}{\varepsilon} \text{ kg/mm}^2.$$

Sprout growth measurement

Measurements of bud length and respiratory rate were made using an infra-red gas analyser (Type Infralyt 4, made in GDR, see section II. Measurement of respiration) regularly every month from March until June 1988 on the same 10 tubers from each treatment of desire variety of potato, stored at 5 °C and 20—25 °C.

Determination of loss in weight

The irradiated and control samples of both varieties of potato were kept in two storage rooms at two different temperatures (5 °C and 20—25 °C). In each storage room 8 lots from both varieties were placed, each containing about 20 kg of potato. In this way 4 lots from each variety (50 Gy, 100 Gy, 500 Gy and control samples) were investigated. All lots of potatoes were weighed monthly and the difference in weight was calculated. First the difference in weight due to respiration and evaporation was calculated. Finally the rotted tubers were removed and the difference in weight due to rotting was also calculated and expressed in accumulated percent. The experimental measurements were taken for 6 months.

Results and discussion

Effect of irradiation and temperature on potato texture

Potatoes of both varieties had good shape, size and colour, the Metal variety especially was large, had a very attractive yellow colour, a good texture and hard body. Changes in texture occur usually after sprouting in potato with concurrent increase in shrinkage and shrivelling. The results of mechanical investigations on potatoes are shown in Figs 1, 2.

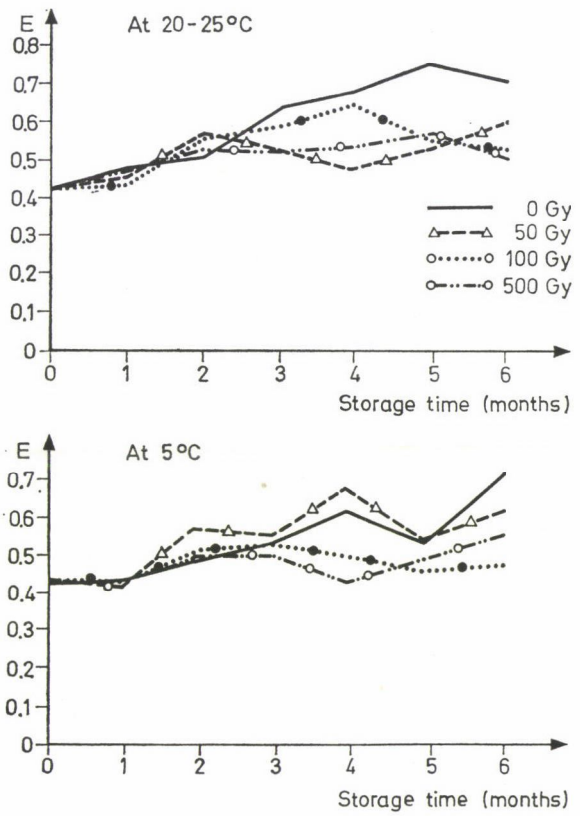

Fig. 1. Effect of irradiation and temperature on the texture of potato *variety Desire* as a function of storage time

It was found that the Young modul as (E) values were increasing in the case of all treatments and control of Desire variety, but in the case of Metal variety slightly decreased or varied. If the Young modul as (E) values are increasing, the deformation is increasing, i.e. the stiffeness is decreasing (I. Huszár personal information). The results obtained indicate that the

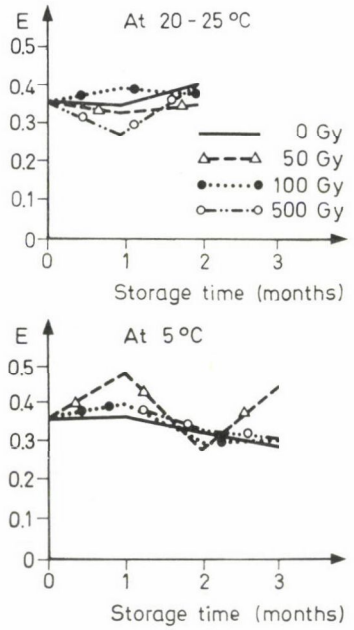

Fig. 2. Effect of irradiation and temperature on the texture of potato *variety Metal* as a function of storage time

unirradiated samples shrivelled, shrank and became gummous during storage, especially those stored at 20—25 °C. They were soft and elastic, because of water reduction and change in chemical composition due to sprouting. The effect of storage, temperature and irradiation (inhibition of sprouting and keeping a good texture quality) on the 100 Gy irradiated samples was similar to the 500 Gy irradiated samples at both storage temperatures, but the samples at 5 °C were much better in texture.

This agrees closely with the results of Cho et al. (1982) who reported that the optimum dose of irradiation used for potato is 125 Gy, which is better in change of texture and extended the storage time by more than 4 months, compared with unirradiated potato at natural low temperature. It also concurs with Beczner's (1983) observation that the texture of control tubers softened as function of storage time and it softened significantly ($P = 0.1—5\%$) more than that of treated tubers, which were better looking and more attractive.

The Metal variety of potato, was very sensitive and deteriorated within 2 months at 20—25 °C and within 3 months of storage at 5 °C. The spoilage was mainly due to fungal rot caused by *Fusarium ventricosum*. It was observed that during further storage all the treatments and control sample of Metal variety showed softening.

Effect of irradiation and temperature on potato sprouting

The results of the present experiment show that the unirradiated stored potatoes sprouted the most rapidly. Sprouting started within 15 days at 20—25 °C storage temperature and within 3 months at 5 °C. The increase in growth of sprouts was quite high in unirradiated stored potato at 20—25 °C. The growth of sprouts in the control samples of Desire variety was linear and gradual at the beginning and then reached a maximum at the end of storage period (Fig. 3).

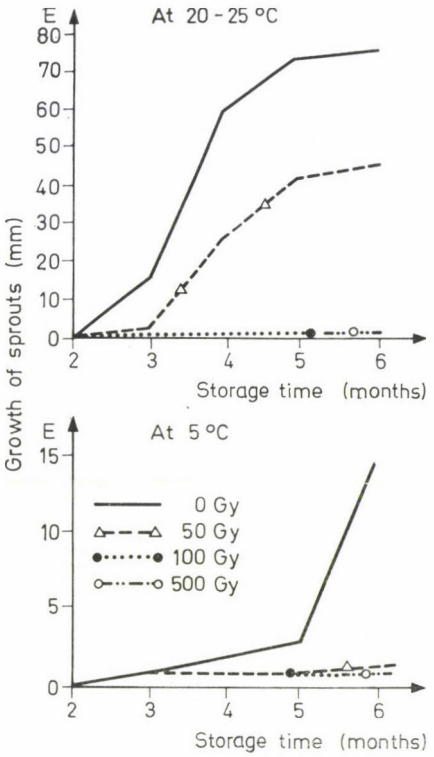

Fig. 3. Effect of irradiation and temperature on sprouts growth rate of potato *variety Desire* as a function of storage time

It was also observed that sprouting started in the irradiated samples of 50 Gy dose stored at 20—25 °C after 2 months of storage, and there was a considerable growth of sprouts at the end of the 6 months storage period. The average length of buds was 45 mm on the 50 Gy treated samples stored at 20—25 °C, while it was 74 mm on the control ones. The 100 Gy and 500 Gy treatments showed only 1 or 2 mm length of buds at the end of storage. These data prove that the 50 Gy treatment is not enough to inhibit potato sprouting at 20—25 °C storage temperature. Some investigators reported that a dose

of 49 Gy or higher was required for good sprout control for most of the varieties of potato (Burton and Hannan, 1957).

Irradiation treatment of 82.5 Gy effectively inhibited sprouting of the tubers at 4.4 °C and 20 °C (Parks et al., 1961). The optimum dose of potato sprout inhibition is 125 Gy until 15—20 days after harvesting and 150 Gy was good for tubers treated later than 45 days after harvesting (Cho et al., 1982). Beczner (1983) found that a dose of 100 Gy irradiation inhibited sprouting, and the loss due to sprouting may be as much as 1—15% for unirradiated potatoes, depending on the variety, year of growth and storage conditions. At 5 °C storage temperature there are less differences in the growth rate between the different treatments, but it was clearly shown that the growth of sprouts on the unirradiated tubers was considerably higher than that on irradiated ones (Fig. 3).

Loss of weight due to respiration and evaporation

It was found that the weight loss of both varieties increased by the storage time at both storage temperatures (5 °C and 20—25 °C), with a concurrent increase in respiration rate of Desire variety control tubers stored at 20—25 °C and of all the treatments and control tubers of variety Metal

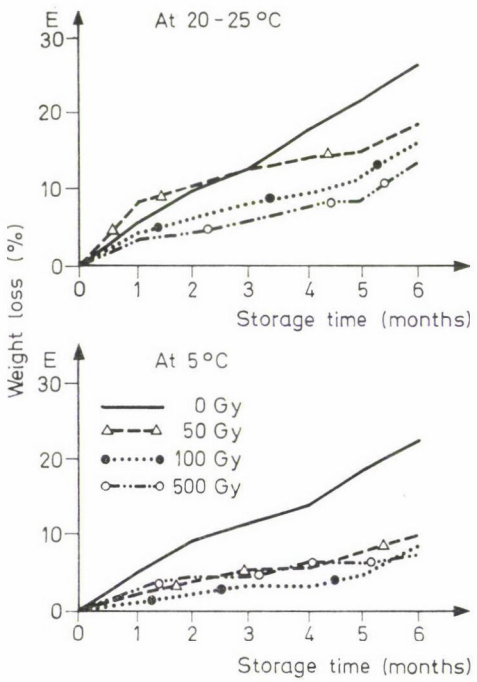

Fig. 4. Effect of irradiation and temperature on the weight loss of potato *variety Desire* in function of storage time. Accumulated weight loss due to respiration

stored at 5 °C. A higher weight loss was found in the control samples than in the irradiated ones. It was 22.1% accumulated percent (total percents of previous months) in the control samples stored at 5 °C, and 25.9% accumulated percent (Total percents of previous months) in the control samples of variety Desire' stored at 20—25 °C, after 6 months of storage (Fig. 4).

In the case of Metal variety the weight loss due to respiration and evaporation was higher in the 50 Gy treatment (32%) and lower in the control tubers (24.4%, accumulated percent) stored at 20—25 °C after 2 months of storage time, and it was also higher in the irradiated tubers of 100 Gy (16.5%, accumulated percent) stored at 5 °C than in the control (9.2%) after 3 months of storage time.

Loss of weight due to rot

The weight loss decreased as the dosage increased in the Desire tubers stored at 20—25 °C.

It started after one month in 100 and 500 Gy treatments, and after 2 months in 0 and 50 Gy treatments. It started after 4 months of storage in 0, 50 and 500 Gy treatments and after 5 months in the 100 Gy treatment stored at 5 °C. Highly significant losses due to rotting were noted (4.2%, accumulated percent) in irradiated tubers of 500 Gy at 5 °C as compared

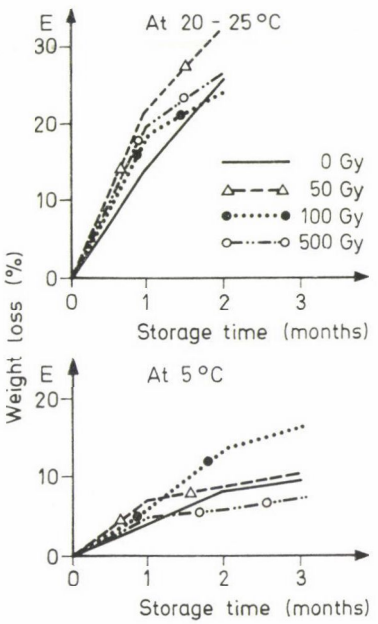

Fig. 5. Effect of irradiation and temperature on the weight loss of potato *variety "Metal"* in function of storage time. Accumulated weight loss due to respiration

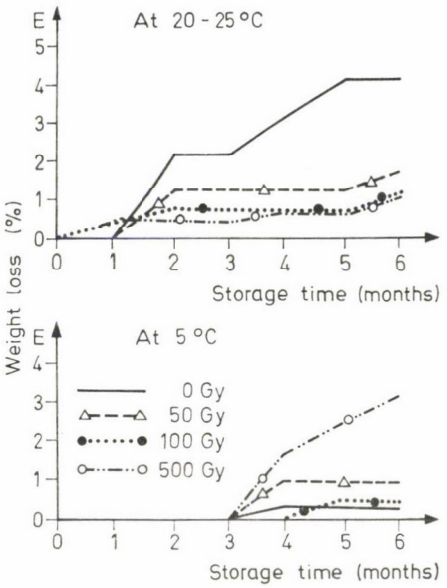

Fig. 6. Effect of irradiation and temperature on the weight loss of potato *variety Desire* as a function of storage time. Accumulated weight loss due to rotting

to the control and 100 Gy treatment (0.32%, 0.44%, accumulated percent, respectively). This is similar to the reports of some investigators such as El-Sayed (1977), Beczner (1980) and Hayashi and Kawashima (1982), while others reported little difference in spoilage between irradiated and control tubers. In the Metal variety tubers the maximum weight loss due to rotting was from 91% to 100% accumulated percent during 3 months of storage at both storage temperatures mainly because of fungal rot caused by *Fusarium ventricosum*.

Conclusion

The control of sprouting by radiation treatment and low temperature resulted in a better texture and low weight losses of irradiated samples stored at 20—25 °C in contrast with excessive and rapidly increasing weight loss exhibited by the unirradiated control samples following the onset of sprouting after a short storage period. The unirradiated samples were shrivelled, shrunken and become gummous during storage, especially those stored at 20—25 °C.

The irradiated tubers stored at 5 °C were much better in texture than those stored at 20—25 °C. The effect of storage temperature and changes in relative humidity play an important role in the shelf-life of potatoes.

Harvesting time, handling and distribution influence the storage life of potatoes.

Irradiation doses of 100 Gy and 5 °C temperature, were found to give the best results in sprout growth inhibition and loss of weight and they produced a better texture quality of potato than the 50 Gy and 500 Gy treatments.

The conclusion is that irradiation treatment can stop the loss due to sprouting, respiration and evaporation, but cannot stop the fungal action after irradiation. The search for varieties suitable for irradiation is necessary. Their physiological and health condition should be "good" or "very good" and the handling of potatoes should be very careful (irradiation and storage

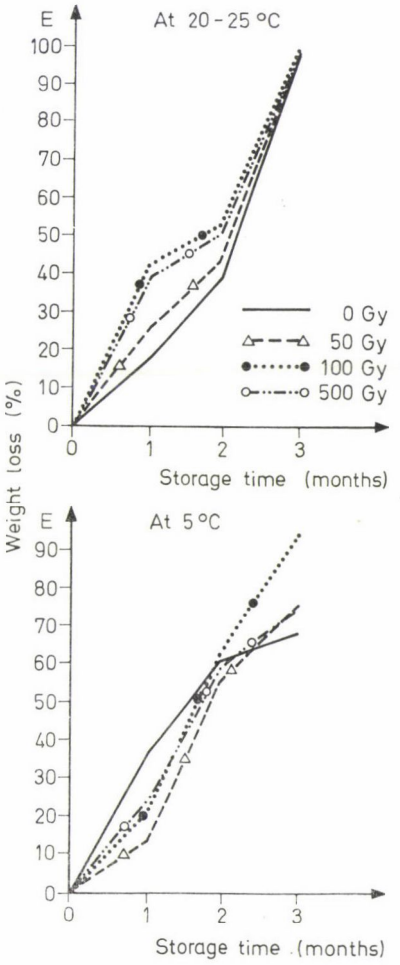

Fig. 7. Effect of irradiation and temperature on the weight loss of potato *variety Metal* in function of storage time. Accumulated weight loss due to rotting

in boxes). This obviates the use of fungicides. If it is unavoidable, the use of a fungicide of natural origin or which breaks down to non-toxic compounds is recommended to minimize the chemical risk to consumers.

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IN VITRO PROPAGATION OF *PHILODENDRON TUXTLANUM* BUNTING WITH BENZYLAMINOPURINE

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The pretreatment of the mother plants — unlike what have been described so far — consisted of the defoliation of the mother plants. Sterilization was carried out with 0.2% $HgCl_2$ and 70% ethanol. The basic culture medium contained the MS macroelements at half concentration, the other components were used at full concentration. For initiation 3 mg/l BA was used. Propagation was divided in two parts: the induction- and the actual phase of propagation. In the former 20 mg/l, then 8 mg/l BA concentrations were applied. Rooting was carried out in vitro, using 0.5 mg/l NAA and 2 g/l active carbon.

Keywords: benzylaminopurine, micropropagation. *Philodendron tuxtlanum.*

Introduction

The *Philodendron tuxtlanum* was described in 1959, and since it proved to be an indoor plant tolerant to the climate of flats, its *in vitro* propagation started in large quantities among the western countries. The first plants were introduced in Hungary through imports, and owing to the increasing demand we began in 1986 to elaborate its *in vitro* propagation. The conventional propagation (with cuttings) is slow, and the sap of the plant causes contact dermatitis (Reffstrup and Boll 1985).

Available literary data concerning the *in vitro* propagation of species from the genus *Philodendron* are relatively few. We therefore took into consideration the literature dealing with the *in vitro* propagation of related genera.

The first data were published by Miller and Murashige (1976), who describe — among others — the propagation of *Syngonium* and *Scindapsus*. Kunisaki (1977) gave an account of the micropropagation of *Scindapsus aureus*, *Ph. oxycardium* and *Ph. lacerum* without, however, giving the cytokinin- and auxin concentrations used. Makino and Makino carried out propagation experiments in 1978 with *Syngonium*, using isopentenyl-adenosine (N^6-iso-penten-2-yladenosiene [2iP]) as cytokinin at concentrations of 3 and 20 mg/l, respectively, but did not present data on the rate of propagation.

Poupet et al. (1983) only published illustrations of the results attained in the micropropagation of several *Phi$_8$odendron* species. Maiae et al. (1983) used benzylaminopurine (6-benzylaminpurine [BA]) as cytokinin at concentrations of 2 and 10 mgl when micropropagating *Ph. scandens* and *Ph. houlletianum*. As auxin they used indolyl-acetic acid (Indole-3-acetic acid [IAA]) at various concentrations. The result obtained was a bunch of 3—4 sprouts. Scaramuzzi and Imbo (1984) described the *in vitro* propagation of two *Syngonium* species with full details of the method of sterilization, the explant, the media used in different phases of propagation and the hormone concentrations. As cytokinin they used BA combined with varying concentrations of different auxins. With the best culture medium combination they obtained 25—35 sprouts from a single bud. Gertsson and Andersson (1985) reported their experiment with *Pl. scandens* where, however, the rate of propagation was very low. Debergh and Maene (1985) supplied detailed data only on the third phase of propagation, though in a paper published in 1981 they referred to root formation observed in the second phase — the propagation phase — of *Philodendron* micropropagation. As cytokinin they used BA and kinetin (Kinetin [K]), as auxin indolyl butiric acid (Indole-3-butiric acid [IBA]).

Since the literary data available so far were insufficient for the propagation technology, we carried out the following experiments.

Materials and methods

The experiments were started in October 1986. The western import mother plant possessed many sprouts which suggested *in vitro* propagation. Two weeks before sterilization the leaves of the plant were removed whereby the bud became visible, and the wounds left at the sites of the petioles healed by the time of sterilization. The advantages of this way of pretreatment were already experienced in the course of the mericlone propagation of *Syngonium podophyllum* (Jámbor-Benczúr, Mándy, Márta, Retkes 1986). Sterilization began with one-hour tap water washing for which Tween 80 was used. After that the lateral buds — with a small piece of stem — were sterilized in 70% ethanol for 5 minutes, then in 0.2% $HgCl_2$ for a further 5 minutes. This was followed by rinsing three times with sterile distilled water, after which the buds were dried in the air current of the laminar box and the stem cut in half was placed onto the starter medium.

In the course of the experiment two kinds of basic culture medium were used. One of them was the Murashige—Skoog (1962) medium (MS); in a part of the experiment its macroelements were only applied at half concentrations (M). The other medium — first used by us (BM) — proved good in the micropropagation of Syngonium, giving a better result than the MS medium (unpublished results).

The composition of the macroelements in BM, the medium used by us, is shown in Table 1. The microelements employed were those of the Heller (1953) combination. The composition of the vitamins we used was already given in our 1988 publication, with the designation BM 3 (Jámbor-Benczúr, Márta, Peredi 1988). The hormone concentrations of the media used in the different phases of propagation are contained in Table 2.

For the solidification of the media 7 g/l agar-agar was uniformly used. As a source of carbohydrate 20 g/l saccharose was added. The value of pH was adjusted with KOH so as to be 5. 6. To the rooting medium (MC) 2 g/l active carbon and 0.5 mg/l naphtyl acetic acid [1-napthtaleneacetic acid (NAA)] were added. The culture medium was sterilized in autoclave at 120 °C, 10^5Pa pressure for 45 minutes.

Table 1

Composition of macroelements in the BM medium

Compound	Quantity mg/l
NH_4NO_3	500
KNO_3	500
$Ca(NO_3)_2 \cdot 4H_2O$	250
$MgSO_4 \cdot 7H_2O$	200
KH_2PO_4	200
Na-Fe EDTA	25
$MnSO_4 \cdot H_2O$	10

Table 2

Hormone concentrations of the culture media used in the experiments

Culture medium			mg/l concentrations of hormones	
BM	MS	M	BA	NAA
BM 6	MS 6		3	—
BM 7	MS 7		5	—
BM 8	MS 8		10	—
BM 9	MS 9		20	—
		M 1	5	—
		M 2	6	—
		M 3	7	—
		M 4	8	—
		M 5	9	—
		M 6	10	—
		M C	—	0.5

The cultures were kept at a 16-hours illumination of 3000 lux and an average temperature of 25 °C.

The cultures were passaged and evaluated at six-week intervals, by counting the sprouts, leaves and roots, and measuring the lengths of the leaves and roots. The experiment was carried out with 4 replications; in each replication 12 buds or groups of sprouts were evaluated.

The results were evaluated by unifactorial random block variance analysis and plotted

Results

With the method described by us 86% sterility could be achieved. With half of the buds induced on BM 6- and the other half on MS 6 medium no difference in development between the two groups was observed. The 1 cm shoots grown from the buds were cut off the old piece of stem and placed onto a BM 9 and a MS 9 medium. Offshoot formation was induced with the

very high BA concentration used here. On the BM 9 medium 25, while on the MS 9 medium 45, offshoots were obtained on an average, from a single initial bud. Therefore, in the induction phase the MS medium was considered more favourable than the BM.

Propagation was started on BM 7 and MS 7 media since in our earlier experiments with Syngonium they proved suitable for this purpose. Propagation on either medium was very limited, but the colonies developed many roots. Therefore, after the removal of the roots the higher BA concentration MS 8 and BM 8 as well as the MS 9 and BM 9 media were used again. On the media No. 9 the plants first became chlorotic then died.

On the MS 8 medium offshoot- and root formation were observed. On the BM 8 medium only offshoots developed.

On the next passage the bunches of offshoots were placed onto the same media as before. On the MS 8 medium the offshoots developed vigorously but the leaves showed signs of chlorosis, while on the BM 8 medium bright green offshoots grew. The rate of propagation with the MS 8 medium was threefold, while in the case of the BM 8 medium fourfold; the latter even gave a significantly better result. In the case of the MS 8 medium the macroelement concentration is thought to have been too high, the chlorosis of the offshoots and the lower rate of propagation may have been its consequences. Therefore, in the subsequent treatments the concentrations of the macroelements were reduced by half.

On comparing the BM 8 and M 6 media no significant difference in the number of offshoots was found. On the M 6 medium the offshoots developed more vigorously and produced larger leaves and several roots.

Owing to the more intensive development of offshoots on the M 6 medium the experiment was continued with this medium.

In our last propagation experiment series the optimum hormone concentration was to be determined, and — if possible — the amount of hormone required for the propagation reduced. For the propagation the M 1—M 6 culture medium series was used. The treatments were set up at three successive points of time and the results of the first two treatments were also evaluated mathematically. The groups of offshoots were always transferred to the same respective media. The results of the third treatment were identical with those of the second, so the detailed evaluation was unnecessary. The results can be seen plotted in Figs 1., 2. and 3.

In the course of the experiments we found that the largest number of offshoots developed on the M 6 medium, but the length of leaf and number of root, on the other hand, were the smallest here. In spite of this, this BA concentration is not the most suitable for propagation, since the colonies became chlorotic after the second passage, though-the unfavourable root formation was minimum. Taking the perfectly healthy development of the

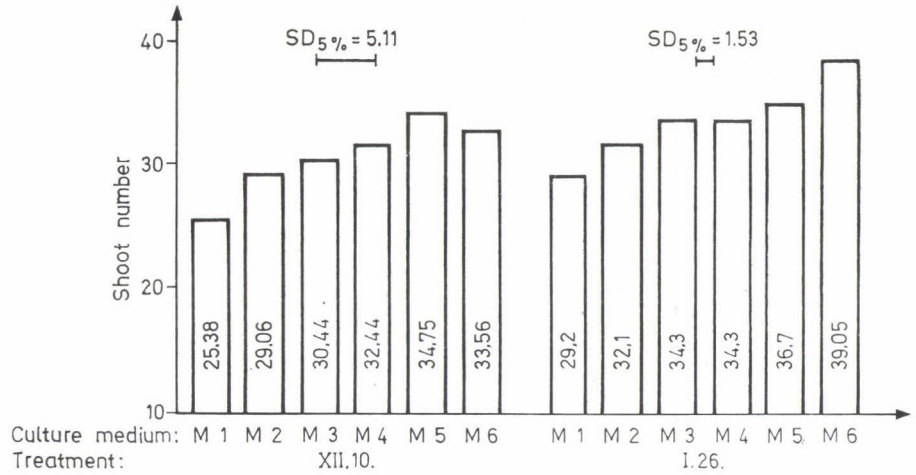

Fig. 1. Trend of the offshoot number of Ph. tuxtlanum as a function of culture medium and time

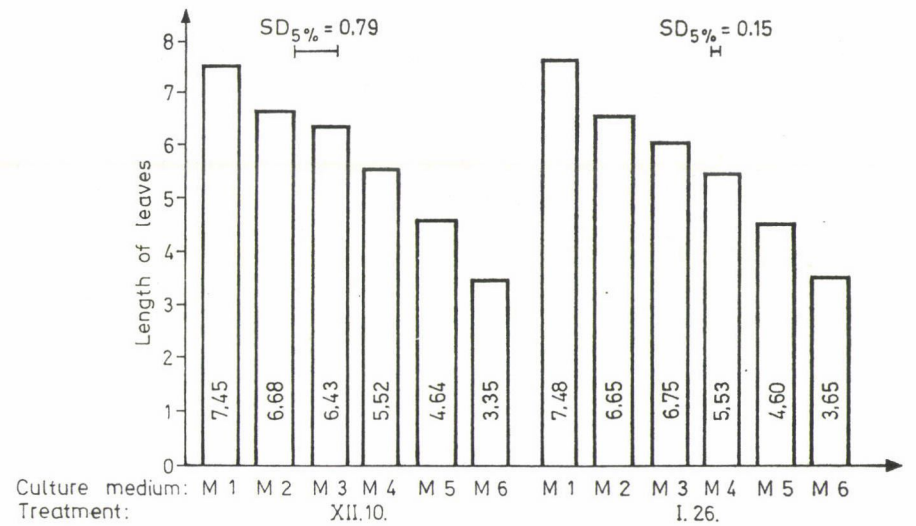

Fig. 2. Length of leaf in Ph. tuxtlanum as a function of culture medium and time

plantlets also into consideration, we found the M 4 medium — containing 8 mg/l BA — to be the most suitable for propagation: the propagation rate attained was fourfold in one passage.

To examine rooting, offshoots produced on the M 1 medium were used. Although on this medium roots developed on some plantlets, they were unsuitable for planting into the soil, as the roots grew upwards into the air. Besides,

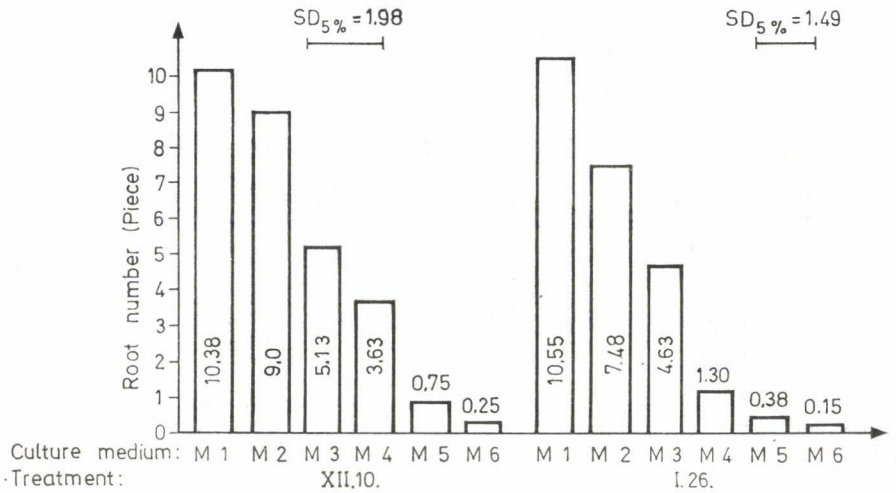

Fig. 3. Root number of Ph. tuxtlanum as a function of culture medium and time

too many offshoots were in one bunch. We cut up the offshoot colonies so that 3—5 plantlets were left in one bunch. The culture medium was completed with active carbon so that the roots developed downwards.

The result of the rooting experiment is shown in Fig. 4. In about a month the roots developed to such an extent that the plantlets could be transplanted to propagation boxes, in glasshouse, in a soil mixture mostly containing peat. After the transplantation the development continued, the survival was 99.5%.

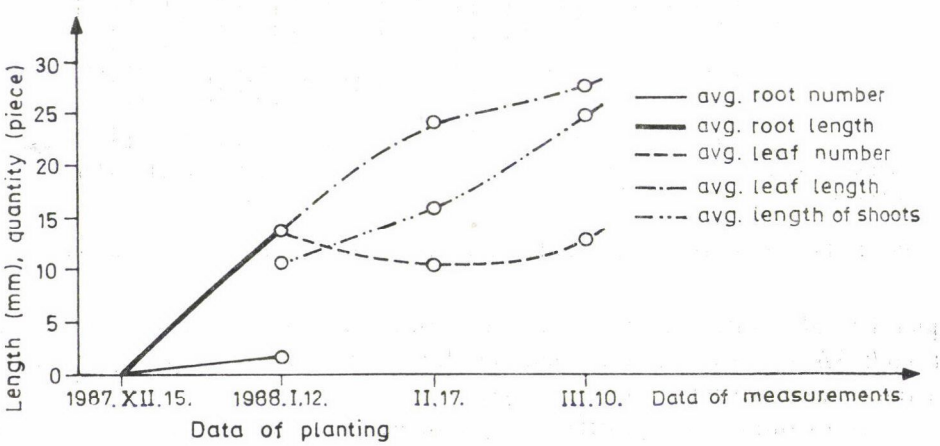

Fig. 4. Development of plantlets in the rooting phase and under the subsequent glasshouse conditions

Conclusions

In the course of the experiments we found that defoliation prior to sterilization had a favourable effect, because the sterilizer could not enter at the site of the wound. This treatment may be called pre-phase which, though, is not the same as the one described by Debergh and Maene (1981).

The sterilization method we used proved good; the buds were not damaged. The hormone concentration of the inducing culture medium is identical with that published by Miller and Murashige (1974) except that we used BA instead of 2 iP.

We divided the propagation in two phases, in the so-called induction (II.a) and the actual propagation (II.b.) phase. In the induction phase a very high — 20 mg/l — BA concentration was used, whereby at the basal part of the small shoot the offshoot formation started. Subsequently, this high concentration proved unnecessary or even harmful, because the offshoots died. The 10 mg/l concentration was sufficient for the propagation; moreover, it had to be further decreased because of the hormone accumulation. In the actual propagation phase 8 mg/l BA proved sufficient. However, it seems probable, that in the case of culturing over a long period even this concentration must be gradually decreased. Root formation in the propagation phase was successfully reduced to minimum.

For rooting the plantlets were taken from a medium containing 5 mg/l BA. The rooting medium contained 0.5 mg/l NAA and 2 mg/l active carbon. This was partly different from what was described by Maene and Debergh (1985). We found that in the II.b. phase too many offshoots formed; therefore, the bunches had to be cut up. As for the amount used of active carbon we followed the above authors, while for rooting we used NAA instead of IBA. In our experiments rooting also took place in vitro, and only subsequently were the plantlets transferred to the peaty mixture of soil.

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GENOTYPE-ENVIRONMENT INTERACTIONS ASSOCIATED WITH MORPHOLOGICAL CHARACTERS OF *BRASSICA*

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Genotype-environment (GE) interaction associated with some morphological characters in two varieties each of swede *(Brassica napus)* and rape *(B. napus)* and four varieties each of kale *(B. oleracea)* and turnip *(B. campestris)* was studied. Six environments were created by sowing seeds at six different times of the year. When the analysis was carried out taking the twelve genotypes together, the mean differences between the genotypes, environment and *GE* interaction components were highly significant for all characters. But a separate analysis of each species detected significant *GE* interaction for only a few characters. *GE* interactions associated with number of leaves in swede; leaf dry weight in rape; and leaf area ratio *(LAR)* and leaf weight ratio *(LWR)* in turnip were linearly related to the environmental indices. A major part of *GE* interactions was lonearly associated with the environmental indices for *LWR* in swede; and total leaf area, number of leaves and hypocotyl dry weight in turnip. Only significant residual mean square values for number of leaves and root dry weight in rape; and leaf dry weight, root dry weight and specific leaf area *(SLA)* in turnip indicated that *GE* interactions were not linearly related to the environmental indices for these characters.

Keywords: environment, genotype, leaf area ratio, leaf weight ratio, regression analysis, specific leaf area.

Introduction

Phenotypic performance reflects the combined influence of genotypic and environmental factors. The usefulness of the phenotype as a predictor of the underlying genotype may be quite variable since, in multi-environmental tests, the genotype may respond differently to various environmental factors. This is called genotype-environment (*GE*) interaction. GE interactions have impeded progress towards a fuller understanding of the control of genetic variation for some time in the past. However, statistical techniques have now been developed which are capable of placing these interactions upon a more rational and hence predictable basis.

The objectives of this experiment were to determine the magnitude of GE interaction variance relative to that of varieties and to investigate the usefulness of the linear regression analysis in Brassica.

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Materials and methods

The subjects were two varieties of swede *(B. napus)* (Rute ∅tofte and Acme), two varieties of rape *(B. napus)* (Lair and Akela), four varieties of kale *(B. oleracea)* (Canson, Proteor, Giganta and THK) and four varieties of turnip *(B. campestris)* (Civasto, Labra, Taronda and Debra). Six environments were created by sowing seeds at six different times of the year. Sowing and harvesting dates were adjusted so that similar growth stage was obtained at each harvest. The sowing and harvesting dates and some meteorological data collected from a nearby meteorological station are given in Table 1. In each environment,

Table 1

The sowing and harvesting dates and some meteorological data

Environment	Sowing date	Harvesting date	Mean air temperature (°C)	Mean sunshine (hr/day)	Mean light intensity (MJ $m^{-2}day^{-1}$
1	1. 2. 78	12. 4. 78	5.9	3.3	8.3
2	17. 4. 78	26. 5. 78	9.2	9.9	16.0
3	31. 5. 78	6. 7. 78	13.2	4.5	13.0
4	11. 7. 78	16. 8. 78	14.0	4.1	14.7
5	18. 8. 78	2. 10. 78	15.0	4.0	10.3
6	6. 10. 78	22. 1. 79	7.2	1.8	3.1

the experiment was replicated 3 times in a randomized block design and there were 10 plants per variety in each replication. Seeds were sown in 12.5 cm plastic pots and randomized on the glasshouse benches of Welsh Plant Breeding Station, Aberystwyth, U. K. The temperature of the glasshouse was controlled in such a way that the minimum was not allowed to fall below 5 °C. In each environment the following measurements were taken: total leaf area per plant, number of leaves per plant, and dry weights of leaf, petiole, stem, hypocotyl and root. *LAR*, *LWR* and *SLA* were calculated from the leaf area and dry weight data.

An overall analysis of variance was conducted for each character, and where the *GE* interaction item was significant, a joint regression analysis as suggested by Finley and Wilkinson (1963) and Bucio Alanis and Hill (1966) was done to separate the linear (heterogeneity of regression mean squares) and non-linear (residual mean squares) components of *GE* interaction. An analysis was carried out separately for each species as well.

Results

Mean squares from the combined analyses of variance of the ten characters studied are shown in Table 2. When the analysis was carried out taking the twelve genotypes together, the mean differences between the genotypes, environment and *GE* interaction components were highly significant for all the characters. Since the twelve genotypes comprised the three species of *Brassica*, genotypic effect and *GE* interaction components were analysed separately for each species treating the two sub-species of napus separately (Table 2).

Swede

The genotypic differences in swede were significant for total leaf area nad *LAR*. The *GE* interaction was significant for number of leaves per plant, *LWR* and *SLA*. Since the analysis of variance can give no further useful information regarding the nature of GE interaction, for each genotype the regression coefficients of individual values on the six environmental means were computed. Following this, the sum of squares measuring the interactions of the genotypes with the environments can be repartitioned into an item measuring differences between the slopes of the two regressions and a residual item which measures the scatter of points about the regression lines. The results of this analysis are also given in Table 2. It is evident that a highly significant proportion of the GE interaction can be ascribed to heterogeneity between the linear regression slopes of the two genotypes for the number of leaves and *LWR*. However, the significant residual MS, as tested by the pooled experimental error, for *LWR* indicated that the departures from the linearity which existed for this character cannot be explained by chance variation. Nevertheless, the linear model would retain considerable predictive value for the number of leaves per plant.

The regression coefficients (b) and the mean values (over environments) of the two genotypes for three characters are shown in Table 3. Since these increments are measured by the means of all genotypes, the average response for any set of genotypes under consideration must have a regression coefficient of unity. A value of $b > 1$ indicates above average response and will be termed sensitive, while genotypes with $b < 1$ indicates below average response and will be referred to as insensitive types. Those genotypes which have average response are usually high yielders in a favourable environment and those with below average response perform relatively better in poor environments. In that respect Rute ∅tofte had below average response for number of leaves, *LWR* and *SLA*. On the other hand, Acme had above average response for number of leaves and *LWR* and average response for *SLA*. Although no correlation coefficients were calculated between the mean performance over the environments and the linear regression coefficients, there was a tendency with all the characters for the sensitive genotypes to have the highest mean performance.

Rape

The analysis of variance revealed that the differences between the two genotypes were significant for all the characters except number of leaves per plant and GE interaction MS was significant for number of leaves, leaf weight and root weight. When GE interaction MS was partitioned it was evident

that the heterogeneity of regression *MS* was non-significant, but the residual *MS* was highly significant, when tested against the pooled error *MS*, for number of leaves and root dry weight. This indicated that the *GE* interactions were not a linear function of the environmental values and no simple relationships existed between the genotypes and the environments. However, for leaf dry weight, the heterogeneity of regression *MS* was highly significant and

Table 2

Mean squares from combined analysis of variance and from the joint

Item	d.f.	Total leaf area per plant (cm^2)	No. of leaves per plant	LAR ($cm^2\ g^{-1}$)	LWR ($g\ g^{-1}$;	SLA ($cm^2 g^{-1}$)
Replication	2	11933	0.71	2171**	0.0041***	2813*
Genotypes (G)	11	485071	76.82***	14311***	0.0244***	19128***
Within swedes (S)	1	133834***	0.20	1713*	0.0023	2025
Within rape (R)	1	131809***	0.60	32041***	0.0194***	54964***
Within kale (K)	3	38999***	2.78**	1169*	0.0033***	4851***
Within turnip (T)	3	33591	20.15**	3623*	0.0027	8589*
Between species	3	1617453***	258.48***	40052***	0.0764***	37699**
Environments (E)	5	574371***	40.97***	30915***	0.0971***	220530***
G X E	55	15586***	3.53***	980***	0.0020***	2316***
S X E	5	9393	1.26*	688	0.0028***	1924*
R X E	5	9304	2.63***	644	0.0003	1733
K X E	15	5066	0.34	270	0.0006	948
T X E	15	18066***	2.36***	685*	0.0011*	1742*
Between species X E	15	27787***	8.95***	2194***	0.0046***	6326***
Heterogeneity between regressions	11	19328***	5.03***	1035***	0.0051***	2161**
Heterogeneity between species	3	23180**	12.19***	978*	0.0127***	2889*
Heterogeneity between varieties within species	8	17883***	2.35***	1057**	0.0023***	1888*
Swede	1	—	3.37*	—	0.0031**	3037
Rape	1	—	1.48	—	—	—
Kale	3	—	—	—	—	—
Turnip	3	33830***	4.38***	1745**	0.0030**	2126
Residual	44	14651	3.15**y	966***	0.0012**	55
Residual swede	4	—	0.73	—	0.0027**	1645
Residual rape	4	—	2.92***	—	—	—
Residual kale	12	—	—	—	—	—
Residual turnip	12	14125**	1.86***	420	0.0006	1646*
Error	110	4928	0.50	331	0.0006	830

*, ** and *** indicate significance level a H 5%, and 0.1% level, respectively.

the residual *MS* was not significant against the pooled error. Heterogeneity of regression *MS* was also significant against residual *MS*, indicating that within each genotype the rate of change of interaction did not vary with the environments. For number of leaves and root dry weight, the sensitive variety Lair had higher mean values. The relationship was reversed for leaf dry weight, where the sensitive variety Akela had the lower values (Table 3).

regression analyses for different characters over six environments

Item	d.f.	Leaf	Dry weight per plant (g)			
			Petiole	Stem	Hypocotyl	Root
Replication	2	0.082	0.0084	0.0031	0.0768	0.0141
Genotypes (G)	11	7.649***	1.18723/8***	0.07923/8***	1.6860***	0.5910***
Within swedes (S)	1	1.027***	0.2600***	0.0073	0.0140	0.0181
Within rape (R)	1	6.341**	2.34263/8***	0.0244***	0.0467*	1.3777***
Within kale (K)	3	0.259*	0.0114	0.0303***	0.0023	0.0250*
Within turnip (T)	3	1.418*	0.0297	0.0090**	0.7037**	0.0531
Between species	3	23.912***	3.4444***	0.2405**	5.4558***	1.6236***
Environments (E)	5	2.249***	0.9818***	0.1847***	0.2351***	0.3271***
G X E	55	0.359***	0.0299***	0.0123***	0.0496***	0.0390***
S X E	5	0.093	0.0088	0.0006	0.0001	0.0032
R X E	5	0.338**	0.0150	0.0041	0.0026	0.0289**
K X E	15	0.062	0.0066	0.0026	0.0006	0.0033
T X E	15	0.328**	0.0174	0.0019	0.0803***	0.0222***
Between species X E	15	0.784***	0.0777	0.0390***	0.1001***	0.1068***
Heterogeneity between regressions	11	0.542***	0.0570***	0.0472***	0.1816***	0.1070***
Heterogeneity between species	3	1.416***	0.1730***	0.1586***	0.4797***	0.3726***
Heterogeneity between varieties within species	8	0.214**	0.0135	0.0054**	0.0698***	0.0074
Swede	1	—	—	—	—	—
Rape	1	1.127***	—	—	—	0.0255
Kale	3	—	—	—	—	—
Turnip	3	0.087	—	—	0.1850***	0.0023
Residual	44	0.314***	0.0230**	0.0036	0.0166	0.0220***
Residual swede	4	—	—	—	—	—
Residual rape	4	0.141	—	—	—	0.0298**
Residual kale	12	—	—	—	—	—
Residual turnip	12	0.389	—	—	0.0541	0.0272
Error	110	0.080	0.0112	0.0020	0.0113	0.0068

*, ** and *** indicate significance level at H 5%, 1% and 0.1% level, respectively.

Table 3

Mean values and regression coefficients (b) for three types of Brassica Swede

Variety	No. of leaves		LWR		SLA	
	b ± SE	Mean	b ± SE	Mean	b ± SE	Mean
Rute ∅ tofte	0.566±0.083	8.85	0.659±0.228	0.613	0.846±0.095	366
Acme	1.434±0.080	9.00	1.341±0.238	0.630	1.154±0.095	380
Rape	No. of leaves		Leaf dry weight		Root dry weight	
	b ± SE	Mean	b ± SE	Mean	b ± SE	Mean
Lair	1.492±0.208	10.72	0.864±0.462	2.427	1.170±0.503	0.787
Akela	0.508±0.298	10.46	1.136±0.442	1.587	0.830±0.573	0.393

Turnip

	Total leaf area		No. of leaves		Leaf dry weight		Hypocotyl dry weight	
	b ± SE	Mean	b ± SE	Mean	b ± SE	Mean	b ± SE	Mean
Civasto	0.871 ±0.276	680	1.018 ±0.188	11.63	1.158 ±0.218	2.460	0.574 ±0.377	0.583
Labra	1.574 ±0.225	713	0.627 ±0.168	12.09	0.779 ±0.305	2.170	0.833 ±0.306	0.483
Taronda	0.695 ±0.153	776	1.016 ±0.138	11.20	0.999 ±0.319	2.833	0.860 ±0.713	0.713
Debra	0.861 ±0.174	756	1.339 ±0.186	13.63	1.064 ±0.135	2.630	1.733 ±0.186	0.940

	Root dry weight		LAR		LWR		SLA	
	b ± SE	Mean	b ± SE	Mean	b ± SE	Mean	b ± SE	Mean
Civasto	1.058 ±0.134	0.557	1.371 ±0.166	159	0.801 ±0.098	0.537	1.194 ±0.106	306
Labra	0.967 ±0.188	0.517	1.001 ±0.159	185	0.935 ±0.070	0.543	1.018 ±0.110	347
Taronda	1.029 ±0.206	0.647	0.851 ±0.099	157	0.978 ±0.062	0.544	0.885 ±0.097	299
Debra	0.947 ±0.157	0.580	0.778 ±0.187	154	1.287 ±0.073	0.517	0.905 ±0.094	307

Kale

Significant genotypic differences were observed for all characters except petiole and hypocotyl dry weights. The *GE* interaction was non-significant for all the characters studied.

Turnip

The analysis of variance indicated significant genotypic differences for number of leaves, *LAR*, *SLA* and leaf, stem and hypocotyl dry weight. Compared to the other three species, *GE* interactions were significant for a larger number of characters. These characters were total leaf area, *LAR*, of the *GE* interactions of those characters was a linear function of the environmental values. For *LAR* and *LWR* only the heterogeneity of regression *MS* was significant when tested both by the pooled error and the residual *MS*. This indicated that each genotype had its own characteristic linear response to environmental change. On the other hand, only residual *MS* was significant for leaf and root dry weights, suggesting that either no relationship or no simple relationship existed between the genotypes and the environments.

The mean values averaged over the six environments and the corresponding regression coefficients (b) for the four genotypes are shown in Table 3. Although no correlation coefficients were calculated between the mean performance and the response to the environments, an indication of the degree of association can be obtained by examining the mean performance and b values for each character. For total leaf area, there was a tendency for the high yielding genotypes to be insensitive and low yielders to be sensitive to the changes in the environment. No regular pattern was observed for the number of leaves and *LAR*.

Discussion

The objective in many plant breeding programmes is the selection of genotypes that are constantly high yielding over the range of environments likely to occur in different locations or seasons. This selection is often inefficient due to *GE* interactions, i.e. the failure of genotypes to have the same relative performance in different environments. Therefore, the importance of quantifying the magnitude of the *GE* interactions in plant breeding programmes cannot be overemphasized.

The results of the present study indicate that *GE* interactions were significant for all the ten characters when the twelve genotypes were included in the analysis. But a separate analysis of each species detected significant *GE* interactions for only 3, 3 and 8 characters in swede, rape and turnip, respectively. None of the characters had significant *GE* interactions in kale. This indicates that the magnitude of the environmental influences on the expression of the different characters of the genotypes varied. Some characters were more highly influenced by the environment than others. The amount by which the expression of an individual character of a genotype is changed in different environments was termed its "phenotypic plasticity" by Bradshaw (1965).

This study has revealed that *GE* interactions associated with the number of leaves in swede; leaf dry weight in rape; and *LAR* and *LWR* in turnip were linearly related to the environmental indices. A major part of *GE* interactions was linearly associated with the environmental indices for *LWR* in swede and total leaf area, number of leaves and hypocotyl dry weight in turnip since the heterogeneity of regression MS was greater than the residual *MS*. For these characters, the linear model retains considerable predictive value for the genotypes concerned, although clearly the model was not entirely satisfactory, since a significant amount of the variation due to *GE* interactions remains unexplained. On the other hand, only significant residual *MS* values for number of leaves and root dry weight in rape, and *SLA*, leaf and root dry weights in turnip indicated that *GE* interactions were not linearly related to the environmental indices for these characters. Therefore, either no relationship or, at least, no similar relationship existed between the genotypes and the environments.

Several studies have shown that the mean performance of a variety over a range of environments and its sensitivity to the environment are highly correlated, but there is considerable evidence to suggest that the two characteristics are under independent genetic control (Parkins and Jinks, 1968a, b). In that respect, the various characters in the present investigation showed differing responses. The sensitive genotypes had either the highest or the lowest mean performance, or no regular pattern was observed.

Knight (1970, 1973) and Witcombe and Whittington (1971) have demonstrated that, where the genotypes in an experiment differ in their physiological response to physical factors in the environment, the linear technique may over-simplify the true response pattern to an extent which could lead to erroneous conclusions. However, in a properly designed experiment this is unlikely, since any differences in physiological response which may occur would be reflected as significant deviations from the fitted regression lines (Hill, 1975). Indeed, if the regression model is satisfactory it is immaterial as to what is the underlying cause of the differential responses as long as the limitations on inferences drawn are appreciated.

The results of the present study have revealed that in some characters of *Brassica GE* interactions can be related to the environment in a simple, linear fashion, thereby providing a measure of predictability. However, further research is needed before the true value of the linear regression technique can be appraised, but its future impact upon the breeding of Brassica is likely to be very considerable.

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BAKING QUALITY OF WHEAT INFLUENCED BY THE STRESS OF GROWING ON SANDY SOIL

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Samples of the wheat variety Jubilejnaja 50 from the sandy areas of the southern part of the Great Hungarian Plain (Zákányszék, Öttömös) have been compared with the samples of Jubilejnaja 50 from the maintenance breeding trials of the Cereal Research Institute regarding their baking industrial and gluten protein data. All the samples derive from the yield in 1987.

The baking property was characterized by the valorigraphic value. The viscoelasticity of the gluten proteins was measured by the gluten spreading. We determined the quantity ratio of protein of gliadin and glutenin type with fractionation by acetid acid, and the protein spectrum of the gluten constituents by SDS-PAGE method.

Unfavourable circumstances (sandy soil, drought) are rather disadvantageous both for quality and quantity. The inherited favourable traits of the wheat variety deteriorate inevitably.

Keywords: wheat, sandy soil, gluten proteins, bread-baking properties.

Introduction

Last year when the wheat crop was brought to the mills, some almost inexplicable phenomena were observed regarding the bread-making quality of the flours. Even in the case of Jubilejnaja 50, which is a variety having good bread-making quality by inheritance, some extremely low quality items occurred in the district plant of the Enterprise for Cereal Trade and Milling, in the Csongrád county. The wheat variety, which regularly has improving quality belonging to the quality group A_1—A_2, had also C_1, and moreover, C_2 items. It led to a loss of prestige for both the producer and the processor.

What could be the reason of the serious degradation of variety?

In order to answer the above question, the series of tests described below have been carried out on some striking items taken over by the district plant in Szeged. Circumstances of production and the correlation between individual physical and chemical traits have been studied.

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** Distric plant of the Enterprise for Cereal Trade and Milling, county Csongrád.

Materials and methods

Daily average samples from partial samples of items from two places of production were taken into the series of examinations. All of the samples derived from the variety Jubilejnaja 50.

The flour of the sample of the wheat variety Jubilejnaja 50 from the maintenance breeding trials of the Cereal Research Institute (CRI) Szeged in 1987 has been regarded as standard.

In Table 1 the places of production and the physical characteristics of the samples are noted. The following data were given by the producers concerning the production conditions.

Table 1

Place of production and physical characteristics of the tested samples

Sample		Hecto-liter weight	H_2O content (%)	Purity (%)	Un-millable (%)	Millable (%)	From millable			
No.	Place of production						Rye (%)	Broken kernels (%)	De-creased value kernels (%)	Others
6	Zákányszék Egyetértés MGTSZ	81.1	12.8	89.2	0.5	10.8	0.4	0.9	6.8	2.2
15	Zákányszék Egyetértés MGTSZ	79.9	12.8	89.1	0.6	10.9	0.4	1.3	6.0	2.6
21	Öttömös Magyar L. MGTSZ	78.5	12.8	83.1	0.7	16.9	1.6	1.8	9.4	5.0
22	Öttömös Magyar L. MGTSZ	78.8	12.8	82.7	0.6	17.3	1.4	1.9	9.3	5.5
30	Öttömös Magyar L. MGTSZ	80.5	12.8	84.0	0.7	16.0	1.3	1.2	9.2	3.9
53	Öttömös Magyar L. MGTSZ	78.2	12.8	84.6	0.5	15.4	1.0	0.9	12.0	2.0
60	Öttömös Magyar L. MGTSZ	81.1	12.9	83.4	0.5	16.6	1.0	0.8	12.4	2.9

Loose wind-blown sand can be found at each place of production. Boundness number is K_A 27—34 in Öttömös on the basis of the tests of the Plant Variety Protection Station, while in Zákányszék the humus content is below 0.25%. The preparation of soil in autumn was arranged in due time and order at both places. In October the 26—32 cm deep ploughing was followed by harrowing, sowing and field-rolling. The quantity of the required fertilizer was calculated on the basis of coil tests results. Potassium was applied as top-dressing in Zákányszék in 90—100 kg/ha and in Öttömös in 120 kg/ha effective material. Herbicides were spread once before shooting. In Zákányszék "DIKORNIT" and in Öttömös "DIKOTEX 40EC" were used.

Maize and barley, and maize for silage and sunflower, respectively, were grown as precrops on the areas under examination.

The seed of Jubilejnaja 50 planted in Zákányszék was certified seed grade II sold by the Seed Trade Enterprise. In Öttömös seed of the same origin was planted. Moreover, their own seed was planted, too, which was tested by the Institute for Plant Production and Qualification in Békéscsaba and graded as good quality seed. It belonged to the multiplication level III. Both places of production are located near each other and their climate was very unfavourable during the summer of 1987. A dry spring was followed by continuous rain for

two weeks, resulting in internal water. Then it turned warm suddenly and the development of the kernels was prevented and the ears dessicated due to summer drought. The crop was harvested in the middle of July.

The wheat yielded 26 q/ha in Zákányszék and 22 q/ha Öttömös in 1987. As a comparison, the yield of Jubilejnaja 50 was normally 56.1 q/ha.

Changes in Technological Value

The tests evaluating the bread-making qualities have been carried out by the Quality Testing Laboratory of the district plant of the Enterprise for Cereal Trade and Milling, Szeged (Karácsony 1970). In Figure 1 the valorigrams and in Figure 2 the numerical values of valorigraph are given. The dough characteristics have become rather unfavourable as compared to the sample from CRI, despite the fact that the same variety has been used. Quick

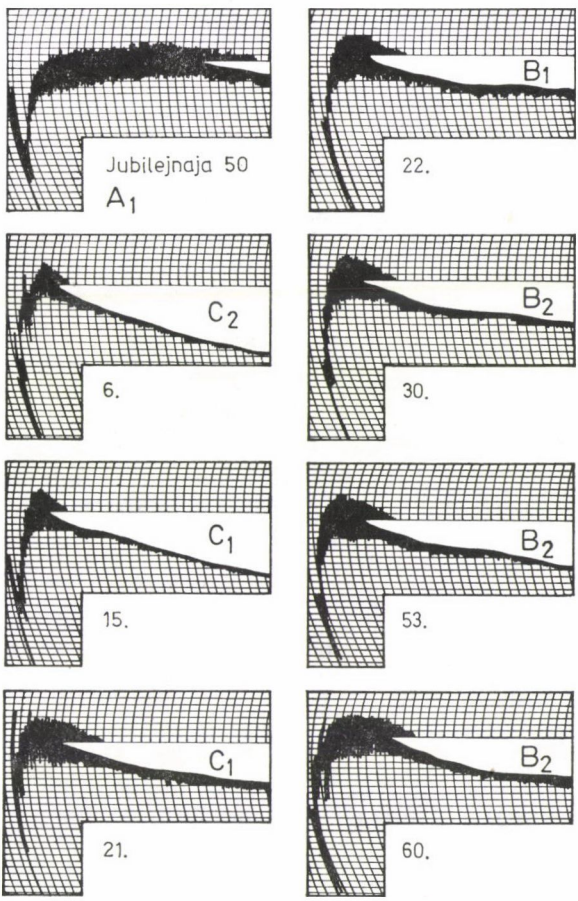

Fig. 1. Valorigrams of the tested samples

dough formation (1.6—2.1 minutes compared to 8 minutes for the control dough) has been followed by short stability (0.2—2.0 minutes). The gluten skeleton weakened quickly as demonstrated by valorigraphic tolerance values of 120—210 VU. Also, the poor viscoelasticity of gluten has been proved by the high values of extensibility (Table 2).

Table 2

Valorigraphic content values and gluten of the samples

Number of samples	Water absorption capacity (%)	Dough formation time (min.)	Dough stability (min.)	Valorigraphic tolerance (VU)	Valorigraphic value	Valorigraphic value group	Raw protein (%)	Wet gluten content (%)	Gluten extensibility (mm)
1	62.4	8.0	2.2	35	85.3	A_1	14.2	33.5	4.0
6	59.2	1.8	0.8	210	28.1	C_2	10.5	23.0	15.0
15	56 8	2.0	0.3	195	32.6	C_1	11.5	27.0	28.0
21	58.2	1.2	1.2	120	44.8	C_1	11.3	27.0	5.5
22	58.0	2.0	1.2	135	48.8	B_2	11.5	27.0	19.0
30	57.6	1.8	0.7	140	45.0	B_2	10.6	26.8	17.0
53	57.4	1.6	0.9	140	45.0	B_2	10.6	25.1	21.5
60	59.0	2.1	2.0	130	48.4	B_2	10.9	27.8	19.0

Poor dough characteristics may be explained by low raw-protein and gluten content, too (Table 2). It is well-known that the quantitative ration of the proteins that form the gluten complex (gliadins and glutenins) have a great significance in determining the bread-making quality of flours.

Samples have been fractioned by acetic acid. As a result, three protein fractions have been received: albumins and globulins soluble in 0.5 m NaCl; gliadins soluble in 0.1 m acetic acid; glutenin type proteins non-soluble in acetic acid. The last two ones form gluten.

The changes in the quantity of the three fractions are demonstrated in Figure 2. It is remarkable that the quantity of proteins soluble in NaCl and acetic acid does not change depending on the place of production, while the quantity of the fraction non-soluble in acetic acid is widely divergent. In this figure the mass ratio of the gluten proteins non-soluble and soluble in acetic acid for the individual samples is shown. It is well-known that the larger part of the glutenin mass in wheat kernel develops in the last period of maturing. As the same variety has been tested, considering the results of the valorigraphic and gluten physiological tests compared with the composition of gluten protein, it can be concluded that kernel development has not been sufficient for the tested samples.

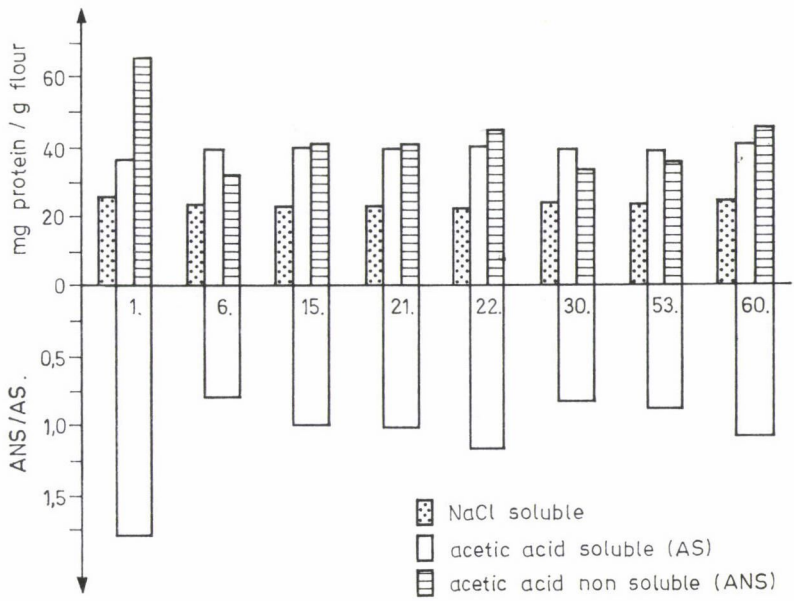

Fig. 2. The quantitative ratio of wheat proteins and the relation of the gluten proteins non-soluble in acetic acid to soluble ones in acetic acid in the samples

Gluten protein tests by SDS PAGE

The traits of gluten proteins on macrolevel are determined by the composition and internations on molecular level. The most simple method is for that purpose the method of Laemmli (1970) called sodium dodecilesulphate polyacrylamid gel electrophoresis (SDS PAGE). Soluble (gliadin type) fractions and, only after reduction, soluble (glutenin type) fractions were extracted by 1 m urea and 2% SDS and 3% -merkapto-ethanol, respectively. The densitograms of the eletroferograms are shown in Figure 3.

As all the samples have been taken from Jubilejnaja 50, one could expect that the subunit composition of the individual gluten proteins according to molecular weight would be nearly similar. However, densitograms have been different (Figure 3). The difference was most striking in the case of the gliadin type protein, where there have not been 67 kD mole subunits in the samples 6 and 15 from Zákányszék. The other parts of the spectrum have been found nearly identical, considering the limits of the reproducibility of the method. On the contrary, in the protein pattern of the glutenin type, specially in high molecular weight (so-called HMW) glutenins (> 68 kD) no significant change has been determined.

Some visually observable differences could be recognized in the subunit ratio of gliadins (the relative largeness of the area below the peak) for the

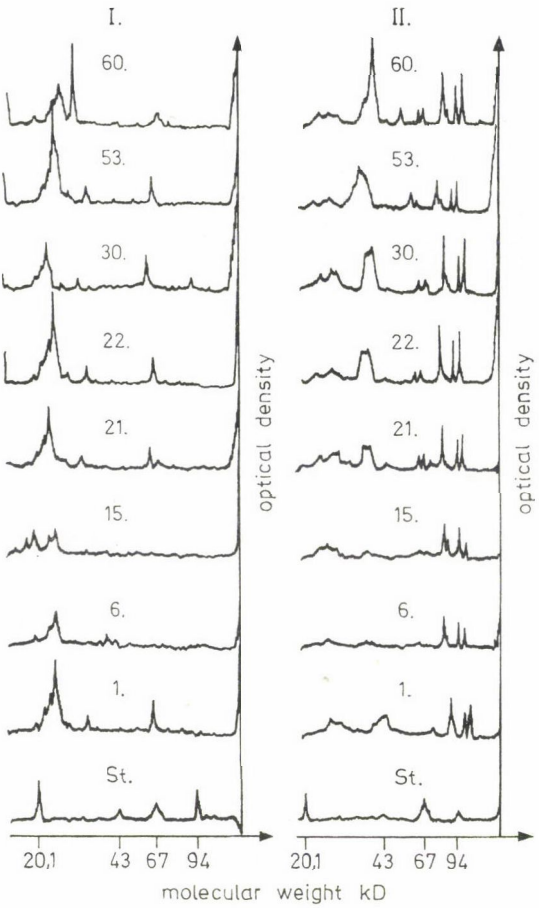

Fig. 3. The molecular weight distribution of gliadin (I) type and glutenin (II) type proteins of the samples tested by SDS PAGE

samples 6 and 15. A certain degree of deviance from variety may have occurred in these samples.

Discussion

On the basis of the results of the dough rheology and baking quality tests, it can be stated that the deterioration of the wheat variety Jubilejnaja 50, which is a good quality wheat by inheritance, has been due to the change in the quantitative ratio of glutenin type proteins, which basically determine the bread-making qualities. Moreover, in the case of the samples 6 and 15 some deviance from the variety traits has occurred, too, which is proved by

the fact that the gluten proteins have been different on molecular level. On the basis of the test results, the conclusion can be drawn that the adverse circumstances have had an important role in the fact that the wheat variety Jubilejnaja 50, being a good quality and high-yielding variety, has yielded far below its capacity. The high quantity and high quality yield is not only the problem of variety. As it is stated by Polhammer (1973), "The inherited good quality of wheat varieties provides only the possibility that the quality characteristic for the variety can develop under the appropriate circumstances.

Nevertheless, if the yield of such varieties has poor quality, the reason does not lie in the variety but in the unfavourable circumstances."

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Plant cultivation

GROWTH DYNAMICS OF GRASSLANDS WITH VARIOUS TIMES OF REGENERATION AND RATES OF NITROGEN

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The seasonal yield of a planted grassland was followed up every 5 days between 10th May and 20th October at 20, 30 and 40 days of regeneration time, 4, 5 and 8 rotations, 50, 67 and 80 kg/ha N per rotation, and with 400 kg/ha N- and 100—200 kg/ha PK active agent applied as basic fertilizer on a year's level.

The growth dynamics of the grassland in dry matter output, plant height and nutrient content was influenced by both the difference in rotation time and the rate of nitrogen fertilization. The increase in dry matter production was most intensive between the 15th and 20th day with the 20-day rotation and between the 25th and 30th day in the case of the 30- and 40-day rotations; the plant height, on the other hand, showed the most intensive growth in the first 5 days.

The daily dry matter production of the grassland is the highest in spring and early in summer and increases with the days of growth; afterwards it is of a lower level and shows a decreasing tendency.

The values of the various nutritive elements change seasonally and with different tendencies. The changes of the N-, P- and K concentrations during the development of the grassland are opposed to the increase in the number of growth days.

Keywords: grassland, fertilization, yield, components.

Introduction

The course of growth of grasses and grasslands must be known if grassland management is to be properly organized. The curve of growth and regeneration of grasslands has an S-shape, and according to Voisin (1968) 3 stages can be distinguished: an initial slow, an intermediary rapid and a final weak growth period.

The growth energy of grasslands shows seasonal changes whereby seasonally different yields are obtained. The growth of grasses relatively fluctuates in the different years as well (Klapp, 1954). In the course of the growth and development of the grassland the quantity and quality of the yield vary in opposite directions (Habibullin, 1977).

The daily production of the grassland — according to Kivimäe (1959) — decreases in the period of stem elongation, then increases again.

In an experiments by Linehan (1947) the daily growth of grass between the 20th and 30th day was nearly as much as between the 1st and 20th day.

Voisin (1968) called the per ha and daily growth or yield of the grassland "performance". The maximum of the performance curve gives the optimum time of rest and simultaneously time shows well the close correlation between time of rest and grass yield: in the case of a short time of rest, the yield will be lower.

Zürn (1953) supplied exact data on the relationship between the seasonal production and the length of the period of rest: a long period of rest resulted in 105% surplus of yield and 41% surplus of crude protein. In another experiment of the same author, the daily growth of grass (kg_8ha) cut every 4 weeks was — except in July — 2.5 times more on average than when cutting was carried out with 2-week intervals. Voisin (1968) established seasonal correlations between time of rest anid daily growth for the countries of Western Europe. He emphasized that the question of grassland fertilization cannot be separated from the complex system of management.

The N-fertilizer increases the growth vigour and performance of the grassland, the daily growth of the grasses (Zürn, 1954). The effect of increasing N-fertilization on the growth dynamics of meadow fescue as manifested in yield and components was studied by Filipek—Kaspercsik (1977).

Materials and methods

Between 1974 and 1980 in a planted grassland at Hajdúszoboszló supplied with annual amounts of 400—100—200 kg/ha NPK active agent we examined the yield, growth and nutrient content of the plant stand every 5 days following 10th May with 20, 30 and 40 days of regeneration time in corresponding 4, 5 and 8 rotations, applying 50, 67 and 80 kg/ha N per rotation. The experiment was laid out in a random block design with 4 replications on plots of 24 m^2 each.

The major analysis data of the soil — a chernozem — in the 0—20 cm layer before the experiment was set up were: pH(KCL) 6.2; K_A 44; total salt % 0.02; humus % 3.4; $NO_3 + NO_2$ 1.7; AL-soluble P_2O_5 44 and K_2O 239 mg/kg.

The treatments of the experiment are shown in Table 1.

Species and variety composition and seed quantity of the grass mixture:

Festuca pratensis Huds	(Szarvasi-54	14 kg/ha
Poa pratensis L. ssp. *latifolia*	("G")	4 kg/ha
Festuca rubra L.	("G")	3 kg/ha
Dactylis glomerata L.	(Szarvasi-51)	8 kg/ha
Bromus inermis Leyss	(Szarvasi-52)	6 kg/ha
Lolium perenne L.	("G-658")	6 kg/ha
Phleum pratense L.	("G")	3 kg/ha
Trifolium repens var. *giganteum* Lagr.		2 kg/ha

During the years of the experiment, the amount of precipitation and the mean temperature did not considerably deviate from the 50-year average (583 mm, 10.0 °C). In the experiment the macroelement content of the grassland was determined with the MÉM NAK methods. The results were evaluated by variance and regression analysis. The yields are expressed in terms of absolute dry matter.

Results

Yield and plant height

According to the data of yearly evaluation (Table 1) the yield and plant height of the grassland increased at a faster rate in the 20-day regeneration treatment due to the greater initial growth vigour, but the total yield fell behind that in the 30—40-day treatments. As regards the annual yield the 30-day rotation gave the most favourable result; in the 40-day treatment the growth was somewhat inferior, because the number of days beyond the opti-

Table 1

Growth dynamics of a grassland with various regeneration times and N doses (1974—1980)

NPK 400—100—200 kg/ha, Period: 10 May—20 October

	Dry matter output kg/ha			Height of grass, cm			Height of grass/day, cm		
Days of regeneration	20	30	40	20	30	40	20	30	40
Number of rotations	8	5	4	8	5	5	8	5	4
N kg/ha/rotation	50	67	80	50	67	80	50	67	80
Days of growth									
5	1454	1027	753	81	59	40	2.0	2.4	2.0
10	2815	2125	1275	93	76	54	1.2	1.5	1.4
15	4483	3473	2402	119	84	70	1.0	1.1	1.2
20	7252	4845	3540	153	98	82	0.9	1.0	1.0
25		6148	4732		112	84		0.9	0.8
30		8527	6597		144	98		0.9	0.8
35			7553			111			0.8
40			8490			125			0.8
S.D ,5%		650			11				
$y = a +$	995+	135+	−442+	78.5−	57.3+	30.0+			
$bx +$	29x+	135x+	181x+	0.66x+	0.65x+	2.45x−			
cx^2	14$\times^2$	4$\times^2$	1$\times^2$	0.22^2	0.71^2	0.003$\times^2$			
R=	0.99	0.99	0.99	0.99	0.99	0.99			

Days of growth	Height per 1 t yield, cm			Yield per 1 cm height kg/ha		
5	55.7	57.4	53.1	18.0	17.4	18.8
10	33.0	35.8	42.4	30.3	28.0	23.6
15	26.5	24.2	29.1	37.7	41.3	34.3
20	21.1	20.2	23.2	47.4	49.4	43.2
25		18.2	17.8		54.9	56.3
30		16.9	14.9		59.2	67.3
35			14.7			68.0
40			14.7			67.9

S. D. refers to the final results of 20, 30 and 40 days.

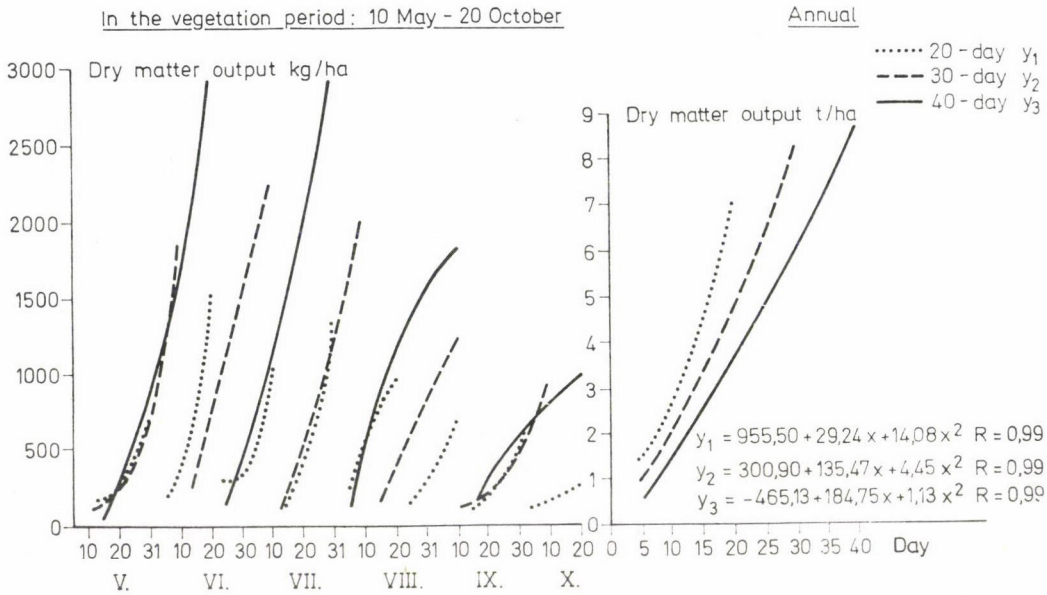

Fig. 1. Growth dynamics of a grassland as expressed in 5—40-day yields per rotation (1974—1980)

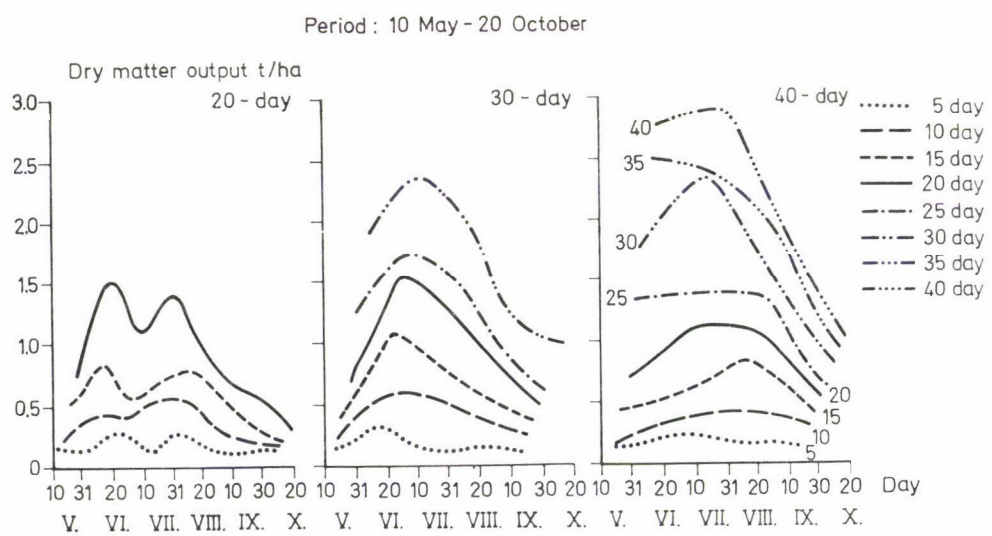

Fig. 2. Growth dynamics of a grassland as expressed in 5—40-day yields per rotation (1974—1980)

mum caused a reduction in yield. The days of growth of the 40-day rotation resulted in a smaller plant height, the value of growth per day being lower. The situation was the same with plant height per 1 t yield, while the value of yield per 1 cm plant height showed the opposite trend.

The growth dynamics of the grassland, the 5-day and annual yield, as well as the height of the plant stand, were also influenced by the different regeneration times and N-doses. On a year's average the growth was most intensive between the 15th and 20th day wth the 20-day rotation, and between the 25th and 30th day with the 30- and 40-day rotations. With the 40-day rotation, the intensity decreased after the 30th day.

The yield data of the grassland for every 5 days during the vegetation period are contained in Table 2. and Figs. 1 and 2. The yield shows seasonal differences and is also influenced by the time of regeneration. In the period examined, the yield of the 20-day rotation was highest in June and July, and so were the differences in yield between the rotations. With the 30 and 40 days of regeneration, the yield level was more even, yet it was the highest

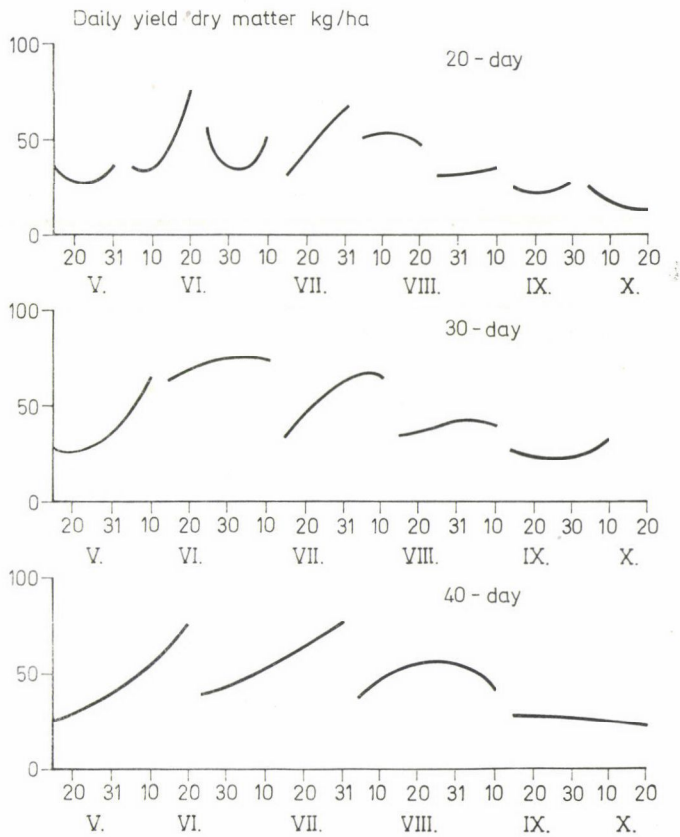

Fig. 3 Performance curves of the grassland per rotation (1974—1980)

Table 2

Yields of a grassland with various regeneration times and N-doses (1974—1980)

Days of reg.	Days of growth	Dry matter output kg/ha by 5 days in the rotations								
		V. 10—31.	VI. 1—20.	VI. 21 VII. 10.	VII. 11—31.	VII. 1—20.	VIII. 21— IX. 10.	IX. 11—30.	X. 1—20.	average
20	5	194	180	277	141	253	157	124	128	182
	10	218	404	397	549	529	286	234	198	352
	15	527	834	529	717	791	532	341	212	560
	20	691	1537	1089	1405	982	688	568	292	907
$y = a +$		132.5+	212.2—	481.0—	63.0+	—79.8+	—10.3+	103.3—	93.5+	125.8+
$bx +$		1.0x+	29.7x+	58.6x+	9.2x+	70.2x—	30.0x+	0.5x+	7.6x+	3.4x+
cx^2		1.4x^2	4.8x^2	4.4x^2	2.8x^2	0.9x^2	0.3x^2	1.2x^2	0.1x^2	1.8x^2
R =		0.97	0.99	0.99	0.99	0.98	0.99	0.99	0.97	0.99

		V. 10—VI. 10.	VI. 11—VII. 10.	VII. 11—VIII. 10.	VIII. 1—20	VIII. 21—IX. 10.	IX. 11—X. 10.	average
30	5	159	331	149	169	131		205
	10	196	620	546	399	249		425
	15	496	1179	821	604	373		695
	20	698	1567	1265	821	494		969
	25	1287	1724	1539	989	609		1230
	30	1882	2342	2064	1237	1002		1705
$y = a +$		235.0—	—75.5+	—129.8+	—39.2+	143.9—		59.1+
$bx +$		29.6x^2	77.7x+	58.4x+	43.3x—	1.7x+		27.2x+
cx^2		2.8x^2	0.03x^2	0.5x^2	0.04x^2	0.9x^2		0.9x^2
R =		0.99	0.99	0.99	0.99	0.99		0.99

		V. 10—VI. 20.	VI. 21—VII. 31.	VIII. 1.—IX. 10.	IX. 11—X. 20.	average
40	5	175	230	203	145	188
	10	177	360	440	298	319
	15	481	654	821	446	601
	20	706	1111	1182	541	885
	25	1330	1360	1385	657	1183
	30	1874	2307	1565	851	1649
	35	2478	2372	1797	906	1888
	40	2772	2858	1774	985	2122
$y = a +$		—59.2+	—119.2+	—286.9+	—14.0+	—110.6+
$bx +$		17.4x+	46.8x+	90.1x—	32.6x—	45.3x+
cx^2		1.4x^2	0.7x^2	0.9x^2	0.2x^2	0.3x^2
R =		0.99	0.99	0.99	0.99	0.99

Table 3

Growth dynamics of a grassland as expressed in yield per day (1974—1980)

Days of reg.	Days of growth	Dry matter output per day, kg/ha								
		V. 10—31.	VI. 1—20.	VI. 21—VII. 10.	VII. 11—31.	VIII. 1—20.	VIII. 21—IX. 10.	IX. 11—30.	X. 1—20.	average
20	5	39	36	55	28	51	31	25	26	36
	10	22	40	40	55	53	29	23	20	35
	15	35	46	35	48	53	35	23	14	37
	20	35	77	54	70	49	34	28	15	45

		V. 10—VI. 10.	VI. 11—VII. 10.	VII. 11—VIII. 10.	VIII. 11—IX. 10.	IX. 11—X. 10.	average
30	5	32	66	30	34	26	41
	10	20	62	55	40	25	43
	15	33	79	55	40	25	36
	20	35	78	63	41	25	48
	25	51	69	62	40	24	49
	30	63	78	69	41	33	57

		V. 10—VI. 20.	VI. 21—VII. 31.	VIII. 1.—IX. 10.	IX. 11—X. 20.	average
40	5	35	46	41	29	38
	10	18	36	44	30	32
	15	32	44	55	30	40
	20	35	56	59	27	44
	25	53	54	55	26	47
	30	62	77	52	28	55
	35	71	68	51	26	54
	40	69	74	44	25	53

Table 4

Growth dynamics of a grassland as expressed in plant height (1974—1980)

Days of reg.	Days of growth	Plant height by 5 days in the rotations, cm								
		V. 10—31.	VI. 1—20.	VI. 21—VII. 10.	VII. 11—31.	VIII. 1—20.	VIII. 21—IX. 10.	IX. 11—30.	X. 1—20.	average
20	5	9	12	12	10	11	10	9	8	10
	10	11	13	13	13	13	11	11	8	12
	15	16	19	17	17	18	12	11	9	15
	20	20	27	23	24	21	17	13	8	19

Table 4 *(co nitnued)*

		V. 10–VI. 10.	VI. 11–VII. 10.	VII. 11–VIII. 10.	VIII. 11 – IX. 10.	IX. 11–X. 10.	average
30	5	9	13	10	11	9	10
	10	11	19	14	13	12	13
	15	16	20	20	16	12	17
	20	19	26	23	18	12	20
	25	25	30	24	20	13	22
	30	31	38	34	25	16	29

		V. 10–VI. 20.	VI. 21–VII. 31.	VIII. 1–IX. 10.	IX. 11–X. 20.	average
40	5	9	11	12	9	10
	10	12	15	15	12	14
	15	17	19	21	13	18
	20	20	26	22	14	21
	25	25	24	23	14	23
	30	29	30	24	15	25
	35	35	35	27	14	28
	20	39	41	31	14	31

Table 5

Growth dynamics of a grassland with various regeneration times and N-doses as

Days of growth	N %			P %			Values of naturitive elements as
	20	30	40	20	30	40	20
							days of
5	3.33	3.24	3.23	0.30	0.30	0.30	2.40
10	3.41	3.32	3.37	0.30	0.29	0.30	2.33
15	3.47	3.49	3.30	0.28	0.29	0.28	2.34
20	3.34	3.31	3.28	0.29	0.28	0.28	2.29
25		3.24	3.25		0.26	0.27	
30		3.11	3.21		0.27	0.27	
35			3.11			0.26	
40			2.93			0.26	
S.D. 5%		0.15			0.01		
$y = a +$	$3.10+$	$3.06+$	$3.18+$	$0.32-$	$0.31-$	$0.31-$	$2.45-$
$bx +$	$0.05x-$	$0.04x-$	$0.02x-$	$0.003x+$	$0.002x+$	$0.002x+$	$0.01x+$
cx^2	$0.002x^2$	$0.001x^2$	$0.0006x^2$	$0.0001x^2$	$0x^2$	$0x^2$	$0.0002x^2$
R =	0.94	0.91	0.97	0.80	0.91	0.97	0.92

S.D. refers to the final results of 20, 30 and 40 days

If the result of the 5th day is 100%, the %s of the further 5-day periods:

10	102	102	104	100	97	100	97
15	104	108	102	93	97	93	98
20	101	102	102	97	93	93	95
25		100	101		87	90	
30		96	99		90	90	
35			96			87	
40			91			87	

in the spring and summer months. In the 40-day treatment at the end of summer there was a decrease in yield after the 35th day.

Yields per day and on that basis the calculated performance curves of the grassland per rotation and for the different regenerations times are shown in Table 3 and Fig. 3. The daily dry matter output of the grassland is higher and of increasing tendency in spring and summer, while at the end of summer and in autumn it is lower and declining.

The seasonal changes in the height of the grassland are shown in Table 4. On a year's average the height increase was most intensive in the first 5 days. However, the rate of the 5-day height increase varied seasonally, by rotation and according to the time of regeneration alike.

Nutrient content

The year's averages are contained in Table 5. In the initial phase of growth (5—15 days) the N-, P- and K content of the grassland is higher;

expressed in the changes of components (1974—1980)

percentages of dry matter							
K %		Ca %			Mg %		
30	40	20	30	40	20	30	40
regeneration							
2.27	2.27	0.41	0.40	0.42	0.22	0.21	0.22
2.36	2.41	0.46	0.44	0.43	0.24	0.21	0.23
2.19	2.29	0.45	0.43	0.42	0.23	0.22	0.22
2.20	2.21	0.45	0.44	0.44	0.23	0.23	0.23
2.21	2.22		0.46	0.45		0.23	0.23
2.20	2.19		0.47	0.45		0.23	0.23
	2.11			0.47			0.24
	2.11			0.48			0.24
0.06							
$2.35-$	$2.35-$	$0.35+$	$0.39+$	$0.42+$	$0.20+$	$0.20+$	$0.22+$
$0.01x+$	$0.003x-$	$0.01x-$	$0.003x+$	$0.002x+$	$0.005x-$	$0.002x-$	$0.0001x+$
$0.0002x^2$	$0.0001x^2$	$0.0005x^2$	$0\ x^2$	$0\ x^2$	$0.0002x^2$	$0\ x^2$	$0\ 0\ x^2$
0.66	0.87	0.91	0.92	0.97	0.77	0.95	0.86
104	106	112	110	102	109	100	105
96	101	110	108	100	105	105	100
97	97	110	110	105	105	110	105
97	98		115	107		110	105
97	96		118	112		119	109
	93			112			109
	93			114			109

Table 6

Growth dynamics of a grassland as expressed in N-content in the case of various regeneration times and N-doses (1974—1980)

Days of reg.	Days of growth	N-content of the grassland by 5 days in terms of dry matter percentage								
		V. 10—31.	VI. 1—20.	VI. 21—VII. 10.	VII. 11—31.	VIII. 1—20.	VIII. 21—IX. 10.	IX. 11—30.	X. 1—20.	average
20	5	2.60	3.22	3.28	3.49	3.43	3.44	3.74	3.42	3.33
	10	2.75	3.35	3.24	3.52	3.63	3.54	3.76	3.53	3.41
	15	2.95	3.37	3.41	3.56	3.67	3.61	3.60	3.61	3.47
	20	3.02	3.16	3.28	3.34	3.36	3.58	3.53	3.44	3.34

		V. 10—VI. 10.	VI. 11—VII. 10.	VII. 11—VIII. 10.	VIII. 11—IX. 10.	IX. 11—X. 10.	average
30	5	2.66	3.07	3.36	3.41	3.71	3.24
	10	2.84	2.99	3.47	3.53	3.77	3.32
	15	3.13	3.39	3.63	3.59	3.73	3.49
	20	2.98	3.12	3.33	3.46	3.66	3.31
	25	2.96	3.08	3.31	3.35	3.50	3.24
	30	2.87	3.01	3.00	3.19	3.46	3.11

		V. 10—VI. 20.	VI. 21—VII. 31.	VIII. 1—IX. 10.	IX. 11—X. 20.	average
40	5	2.86	3.08	3.34	3.63	3.23
	10	3.09	3.08	3.61	3.69	3.37
	15	3.06	3.19	3.36	3.58	3.30
	20	3.07	3.12	3.37	3.57	3.28
	25	3.16	3.16	3.29	3.40	3.25
	30	2.90	3.30	3.23	3.41	3.21
	35	2.75	3.17	3.20	3.29	3.11
	40	2.58	2.91	3.12	3.11	2.93

then, with the senescence of the plants, it decreases. With the result of the 5th day taken for 100%, by the 40th day the macroelement content was reduced to 91% for N, 87% for P and 93% for K. With Ca and Mg the reverse was true: their concentrations increased simultaneously with the number of growth days.

With the N, P and K significant differences were found between the final results of the 20-, 30- and 4-day treatments.

Tables 6—8. give detailed data on changes in the N-, P- and K contents during the vegetation period. These data are important for the feeding of animals. The N content shows an increasing tendency in the course of the vegetation period, but in the 5—40-day span of life of the grassland it decreases.

Table 7

Growth dynamics of a grassland as expressed in P-content in the case of various regeneration times and N-doses (1974—1980)

Days of reg.	Days of growth	P-content of the grassland by 5 days terms of dry matter percentage								
		V. 10—31.	VI. 1—20.	VI. 21—VII. 10.	VII. 11—31.	VIII. 1—20.	VIII. 21—IX. 10.	IX. 11—30.	X. 1—20.	average
20	5	0.30	0.30	0.30	0.31	0.31	0.28	0.31	0.30	0.30
	10	0.30	0.30	0.31	0.32	0.30	0.27	0.31	0.30	0.30
	15	0.28	0.29	0.29	0.30	0.29	0.28	0.30	0.29	0.29
	20	0.27	0.28	0.28	0.30	0.28	0.29	0.29	0.29	0.29

		V. 10—VI. 10.	VI. 11—VII. 10.	VII. 11—VIII. 10	VIII. 11.—IX 10	IX. 11—X. 10.	average
30	5	0.30	0.28	0.33	0.30	0.29	0.30
	10	0.32	0.28	0.31	0.28	0.28	0.29
	15	0.30	0.27	0.30	0.27	0.30	0.29
	20	0.27	0.26	0.31	0.27	0.29	0.28
	25	0.26	0.25	0.28	0.25	0.27	0.26
	30	0.27	0.25	0.27	0.27	0.27	0.27

		V. 10—VI. 20.	VI. 21—VII. 31.	VIII. 1—IX. 10.	IX. 11—X. 20.	average
40	5	0.30	0.28	0.30	0.32	0.30
	10	0.30	0.28	0.30	0.32	0.30
	15	0.27	0.27	0.28	0.31	0.28
	20	0.27	0.28	0.27	0.29	0.28
	25	0.26	0.28	0.26	0.28	0.27
	30	0.25	0.29	0.25	0.27	0.27
	35	0.24	0.29	0.24	0.27	0.26
	40	0.23	0.29	0.23	0.27	0.26

The concentration of P changes during the vegetation period; in summer it is somewhat higher (depending also on the amount of precipitation), then with the senescence of the grassland; in further days of the rotation its value is lower.

The percentage K content of the grassland is the highest at the beginning of the vegetation period; later, and in the 4—50-day lifetime of the grassland, it slightly decreases.

Conclusions

— Under the influence of ecological factors the dry matter output of the grassland gradually decreases from May-June to the end of October, though the seasonal yields are influenced by the time of regeneration and the rate of N-fertilization.

Table 8

Growth dynamics of a grassland as expressed in K-content in the case of various regeneration times and N-doses (1974—1980)

Days of reg.	Days of growth	K-content of the grassland by 5 days in terms of dry matter percentage								
		V. 10—31.	VI. 1—20.	VI. 21— VII. 10.	VII. 11—31.	VIII. 1—20.	VIII. 21— IX. 10.	IX. 11—30.	X. 1—20.	average
20	5	2.73	2.44	2.41	2.40	2.36	2.30	2.27	2.28	2.40
	10	2.69	2.38	2.38	2.38	2.28	2.20	2.20	2.16	2.33
	15	2.68	2.33	2.33	2.36	2.37	2.22	2.17	2.24	2.34
	20	2.60	2.33	2.23	2.36	2.35	2.11	2.14	2.22	2.29

		V. 10—VI. 10.	VI. 11—VII. 10.	VII. 11— VIII. 10.	VIII. 11—IX. 10.	IX. 11—X. 10.	average
30	5	2.59	2.33	2.18	2.15	2.10	2.27
	10	2.70	2.39	2.27	2.23	2.19	2.36
	15	2.52	2.23	2.07	2.09	2.05	2.19
	20	2.52	2.25	2.10	2.11	2.03	2.20
	25	2.50	2.28	2.11	2.10	2.05	2.21
	30	2.48	2.25	2.11	2.10	2.05	2.20

		V. 10—VI. 20.	VI. 21—VII. 31.	VIII. 1—IX. 10.	IX. 11—X. 20.	average
40	5	2.64	2.36	2.07	2.02	2.27
	10	2.79	2.41	2.24	2.21	2.41
	15	2.43	2.33	2.23	2.18	2.29
	20	2.38	2.24	2.14	2.09	2.21
	25	2.38	2.25	2.13	2.10	2.22
	30	2.36	2.22	2.10	2.08	2.19
	35	2.27	2.11	2.03	2.01	2.11
	40	2.27	2.10	2.04	2.01	2.11

— The growth dynamics of the grassland — within a given phase — is the highest in the first period. The optimum regeneration time is mostly 30 days and, in the case of 40 days, occasional reduction of yield may occur.

— The N- and P-concentrations gradually increase, while the K content decreases during the vegetation period.

— With the senescence of the plants the N-, P- and K-contents show a decreasing tendency during the P-40 days examined, while the Ca- and Mg-concentrations increase.

— The yield and quality of the grassland show seasonal changes during the vegetation period. There is a negative correlation between the quantity of yield and the values of several nutritive elements, which must be taken into consideration if the grassland is to be properly managed.

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EFFECT OF NITROGEN AND PHOSPHORUS ON YIELD AND QUALITY OF SUGAR BEET IN SALINE-SODIC SOILS

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A field experiment was conducted to study the effect of nitrogen and phosphorus doses on the yield and quality of sugar beet in saline-sodic soils. The results showed that the application of increasing rates of nitrogen and phosphorus increased root and sugar yields. Nitrogen decreased, while phosphorus increased, the sucrose content in roots. Applications of nitrogen increased, and phosphorus decreased, nitrogen in leaves and roots. Phosphorus in roots and leaves increased with increasing doses of nitrogen (up to 120 kgN) and phosphorus (up to 60 kg P_2O_5). Sugar beet corps reduced the pH of the soil. The reduction in pH of soil increased with increasing doses of nitrogen and phosphorus. It is concluded that 120 kg nitrogen and between 60 to 90 kg P_2O_5 were optimum for higher yields and a good quality of sugar beet in such soils.

Keywords: sugar beet, nitrogen, phosphorus, saline-sodic soils, yield, quality.

Introduction

Kanwar (1957) suggested the possibility of introducing the sugar beet in the saline-sodic soils of India. Deficiency of nitrogen and phosphorus is a most common occurrence in saline-sodic soils. The inadequate supply of nitrogen limits root yield, while the excess stimulates top growth and reduces sugar percentages in the roots of sugar beet (Ulrich 1942). Applications of phosphorus have been found to increase root yields and sucrose, and to counteract the adverse effect of excess nitrogen. In this investigation an effort has been made to discover the optimum nitrogen and phosphorus requirements of sugar beets in saline-sodic coils.

Materials and methods

The sugar beet is recommended for Northern India, where the summer is hot and winter is cold. It is sown from October to November, when temperatures begin to decline and harvested in the months of April/May when temperatures rise. The minimum temperature at sowing is around 10 °C and the maximum temperature during harvesting is around 35 to 40 °C. Average minimum and maximum temperatures from November to February lie

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between 5 and 25 °C. The average rainfall of the experimental area, which occurs mainly between July and September, is 840 mm, the remainder of the year is mostly dry, and well or canal water is used for irrigation.

Nitrogen and phosphorus doses as per treatment details in Table 1 were added to experimental plot (4 × 3 m) in the form of urea and single superphosphate. Half of the nitrogen and full doses of phosphorus were applied as basal doses and the remaining half of nitrogen was top dressed at the time of thinning. Treatments were replicated three times. General properties of the experimental soil are given in Table 2. pH of the soil was determined, in 1 : 2 soil : water suspension, by a glass electrode. Sugar beet (var. Ramonskaya) seeds were dibbled at row to row and plant to plant spacings of 50 and 20 cm, respectively; and plant density at harvest was around 70000/hectare. Crops were irrigated as and when required, and harvested at the proper time. Beet roots were cleaned and weighed.

Table 1

Nitrogen and phosphorus treatments

Treatments		Rate kg nutrient per hectare	
		N	P_2O_5
N_0	P_0	0	0
N_0	P_{30}	0	30
N_0	P_{60}	0	60
N_0	P_{90}	0	90
N_{60}	P_0	60	0
N_{60}	P_{30}	60	30
N_{60}	P_{60}	60	60
N_{60}	P_{90}	60	90
N_{120}	P_0	120	0
N_{120}	P_{30}	120	30
N_{120}	P_{60}	120	60
N_{120}	P_{90}	120	90
N_{180}	P_0	180	0
N_{180}	P_{30}	180	30
N_{180}	P_{60}	180	60
N_{180}	P_{90}	180	90

Table 2

General characteristics of soil

Characteristic	Value
Texture	Silty loam
Organic carbon (%)	0.40—0.66
pH (1 : 2, Soil : water)	9.0 to 9.5
EC (1 : 2, soil : water) dS/m	0.89 to 1.11
C.E.C. me/100 g	27.2
Exchangeable sodium me/100 g	7.6
Exchangeable sodium percent	28.5

Soil samples were taken before sowing and after the harvest of crops whereas leaf and root samples were taken at the harvest. Soil samples were taken with soil sampling tube auger. All the leaves (including petioles) of sampling plants were dried, and thoroughly mixed. For the collection of root samples eight to ten whole roots were randomly selected from each

plot and washed. Equal amounts from each root (in a proper proportion from all parts of it) were crushed into brei by a crusher. The brei was thoroughly mixed and the juice (after pressing) from representative samples was used for determination of total soluble solids with the refractrometer. For the extraction of juice for sucrose determination, 26 g of brei samples were mixed with 177.7 ml of lead acetate reagent and blended in a blender for 30 seconds. The content was filtered and sucrose per cent in the filtrate was determined by the polarimeter. Sugar yield was calculated by the formula:

$$\frac{\text{root yield} \times \text{sucrose per cent}}{100}$$

and purity per cent was calculated as

$$\frac{\text{sucrose per cent}}{\text{T. S. S.}} \times 100$$

Crushed root samples were dried in an oven at 60—70 °C, ground and mixed thoroughly. A portion of the leaves and root samples was analysed for total nitrogen (*Kjeldahl* method) and phosphorus (in triacid digested mixtures by vanadomolybdate yellow colour method). All data were subjected to an analysis of variance and the significance of data was determined by "F" test.

Results and discussion

Root yields

Increasing applications of nitrogen from 0 to 180 kg N and phosphorus from 0 to 90 kg P_2O_5 increased the yield significantly (average values) from 9.79 to 23.79 t/ha and from 11.79 to 21.62 t/ha, respectively (Table 3). The results also showed that both the elements were complementary to each other, as in the absence of one element (N or P) there was poor response to another.

Root yields and sucrose content are low as compared to European countries. The main reason for low yields in India is high temperatures during maturity which retard the greater accumulation of carbohydrates in the roots as compared to low temperatures and long duration of crops in European countries. The reason for low purity in the present experiment is the presence of high sodium in sodic soils which increases impurities in roots.

Narwani and Shekhawat (1982) reported that the average root yields of the commercial sugar beet area in India varied between 20 to 30 t/ha. Therefore, the roots yields obtained with the addition of 120 or 180 kg N and 90 kg P_2O_5 (around 30 t/ha) even in the saline-sodic soils were as good as obtained in normal soils.

Sugar yield

At all levels of nitrogen and phosphorus the sugar yield increased significantly with the increase of nitrogen and phosphorus doses. The increase in total sugar yield was mainly due to the increase in root yield (Table 3).

Table 3

Effect of nitrogen and phosphorus on yield and quality of sugar beet in saline-sodic soils

Treatments	Yield t/ha		T.S.S. %	Sucrose %	Purity %
	Root	Sugar			
N_0 P_0	8.81	1.248	21.8	14.15	65
N_0 P_{30}	9.37	1.395	22.3	14.87	68
N_0 P_{60}	10.37	1.549	21.8	14.92	69
N_0 P_{90}	10.62	1.601	23.2	15.05	66
N_{60} P_0	9.75	1.344	21.8	13.90	64
N_{60} P_{30}	10.68	1.590	22.3	14.90	67
N_{60} P_{60}	13.00	2.041	22.9	15.55	70
N_{60} P_{90}	16.06	2.343	22.1	14.60	66
N_{120} P_0	14.31	1.918	20.7	13.37	65
N_{120} P_{30}	16.50	2.309	20.3	13.97	68
N_{120} P_{60}	19.56	2.972	21.2	15.17	74
N_{120} P_{90}	28.62	4.574	21.4	15.95	75
N_{180} P_0	14.31	1.882	21.6	13.15	59
N_{180} P_{30}	22.81	3.604	19.6	15.82	79
N_{180} P_{60}	25.26	4.082	21.0	15.95	75
N_{180} P_{90}	31.18	5.100	19.4	16.35	86
Averages					
N_0	9.79	1.449	22.3	14.75	67
N_{60}	12.39	1.830	22.1	14.74	67
N_{120}	19.75	2.943	20.9	14.62	70
N_{180}	23.39	3.668	20.4	15.32	75
P_0	11.79	1.599	21.5	13.64	63
P_{30}	14.84	2.224	21.1	14.89	71
P_{60}	17.05	2.661	21.5	15.40	72
P_{90}	21.62	3.404	21.5	15.48	73
C.D. at 5% for N and P	1.89	0.303	1.2*	0.35	5
for N×P	3.78	0.607	2.4	0.67	NS

* Only for N; NS-nonsignificant

Total soluble solids (T. S. S.)

Total soluble solids decreased with the increase of nitrogen application. The decrease was, however, significant, only at 120 kg N. Maximum and significant effects of increasing level of nitrogen were observed at 90 kg P_2O_5. Applications of higher amounts of nitrogen have been found to reduce T. S. S. in roots by Sinner et al. (1976) and Halvorson and Hartman (1980).

Sucrose

Higher sucrose contents in roots were observed at higher rates of N (180 kg) and P (60 and 90 kg). The data (Table 3) also showed that, in the absence of P, sucrose decreased with increacing N application; whereas with

increasing levels of P, sucrose increased with increasing N application. This indicated that sucrose content increased with increasing doses of phosphorus. Correlation coefficients (0.6687) between total P uptake and sucrose was positive; whereas there was a negative correlation between sucrose per cent and the per cent N in roots (—0.3606). Many workers (Cheema 1973, Stanacev and Pavlovic 1980) reported that applications of N had depressive effects on sucrose contents of roots; and Abbott and Nelson (1983) indicated that sucrose increased with increasing levels of P.

Purity

Purity of juice increased with increasing doses of nitrogen and up to 30 kg phosphorus. At low nitrogen levels the yields were very low and probably the proportion of impurities were higher; whereas at higher doses the yields increased, the impurities were diluted and, hence, the higher purity. It was also found that, at high N and P doses, sodium content in roots decreased and this may be another reason for higher purity at high nitrogen and phosphorus levels.

Nitrogen content and total uptake

Nitrogen contents in the leaf and roots increased with increasing levels of nitrogen in the soil (Table 4). At any nitrogen dose the nitrogen content in the leaf decreased with increasing phosphorus doses in soil. On the contrary, the total N uptake increased with increasing levels of both N and P. There was high correlation (0.9109) between total N uptake and root yields. These results thus revealed that decreases in N content, with increasing P doses, was mainly due to the increase in yield, resulting in a dilution of N in roots and leaves.

Phosphorus content and total uptake

Phosphorus contents in the roots and leaves increased with increasing dose of N up to 120 kg, and P up to 60 kg, but the total P uptake was higher at higher doses of N and P (Table 4). Draycott and Durrant (1976) also observed a similar increase in phosphorus content (0.05%) in tops and roots with fertilizer application. Athar (1972) showed that nitrogen and phosphorus contents of the petioles increased with the application of nitrogen and phosphorus to the crop, regardless of the method of application. There was a very high correlation (0.9799) between the total P uptake and root yields. Therefore, the higher P uptake was mainly due to higher yields at higher rates of N and P.

Table 4

Effect of nitrogen and phosphorus doses on nitrogen and phosphorus content and uptake by sugar beet and pH of saline-sodic soils

Treatments	N (%)		P (%)		Total N uptake kg/ha	Total P uptake kg/ha	pH
	leaf	root	leaf	root			
N_0 P_0	1.60	1.29	0.282	0.142	27.5	3.67	9.00
N_0 P_{30}	1.89	0.99	0.289	0.156	23.9	3.71	8.89
N_0 P_{60}	1.85	0.82	0.371	0.168	25.9	5.29	8.83
N_0 P_{90}	1.62	0.83	0.378	0.184	24.2	4.81	8.71
N_{60} P_0	2.54	1.45	0.321	0.177	33.6	4.15	8.91
N_{60} P_{30}	2.43	1.35	0.337	0.220	36.5	5.60	8.75
N_{60} P_{60}	2.22	1.28	0.364	0.259	39.0	7.30	8.63
N_{60} P_{90}	2.13	1.16	0.372	0.267	38.8	7.98	8.50
N_{120} P_0	2.83	1.60	0.350	0.180	52.3	6.07	8.74
N_{120} P_{30}	2.27	1.54	0.368	0.222	50.5	7.53	8.54
N_{120} P_{60}	2.04	1.33	0.389	0.277	48.6	10.02	8.40
N_{120} P_{90}	1.81	1.23	0.393	0.279	65.6	14.82	8.14
N_{180} P_0	2.92	1.63	0.363	0.238	50.2	7.01	8.52
N_{180} P_{30}	2.42	1.52	0.379	0.223	66.1	9.85	8.45
N_{180} P_{60}	1.82	1.36	0.385	0.244	66.9	12.34	8.29
N_{180} P_{90}	1.85	1.29	0.382	0.249	67.1	13.90	8.18
Averages							
N_0	1.74	0.98	0.329	0.163	25.4	4.37	8.86
N_{60}	2.33	1.31	0.349	0.231	37.0	6.26	8.70
N_{120}	2.24	1.42	0.373	0.239	54.2	9.61	8.46
N_{180}	2.28	1.43	0.378	0.238	62.7	10.77	8.37
P_0	2.47	1.49	0.330	0.184	40.9	5.22	8.78
P_{30}	2.25	1.35	0.343	0.205	44.2	6.67	8.66
P_{60}	2.01	1.20	0.378	0.237	45.1	8.74	8.54
P_{90}	1.85	1.11	0.381	0.245	49.1	10.38	8.39
C.D. at 5% for N + P	0.09	0.05	0.007	0.007	4.4	0.83	0.10
for N×P	0.18	0.10	0.015	0.015	—	—	0.20

pH of soil

There was a reduction in pH of soil with the increasing N and P doses. Very high negative correlations (—0.9436) between pH and root yields indicated that sugar beets reduce the pH of the sodic soil. Sugar beet is highly resistant to high sodium and removes a large amount of it (50—100 kg) even under normal soil conditions (Draycott et al. 1972). Therefore, the reduction in the pH of soil is probably due to the removal of sodium from soil.

It can be concluded that 120 kg nitrogen and between 60 to 90 kg P_2O_5/ha were optimum for higher yields and a good quality of sugar beet in saline-sodic soils. Sugar beet also reduced the pH of sodic soils.

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EFFECT OF POPULATION ON NUTRIENT UPTAKE OF PIGEONPEA GENOTYPES IN SOLE AND INTERCROPPED SITUATION WITH SORGHUM CO 22

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A field experiment was conducted during the kharif season, 1984, to study the effect of plant population and intercropping of sorghum CO 22 with two genotypes of pigeonpea SAI and CO 5. Increase in plant population of SA 1 up to 60,000 plants ha^{-1} increased the nitrogen, phosphorus and potassium uptake.

The uptake pattern decreased with an increase in pigeonpea population. In CO 5, uptake of nitrogen, phosphorus and potassium increased with an increased plant population from 74,000 to 1,48,000 plants ha^{-1}. Intercropping of sorghum CO 22 reduced the nutrient uptake of pigeonpea genotypes at all population densities.

Keywords: Intercropping sorghum CO 22, population levels of pigeonpea, pigeonpea genotypes, sole pigeonpea, CO 5, SA 1.

Introduction

Information on nutrient uptake by pigeonpea genotypes under different plant population densities under intercropping situations are lacking. Hence this study was taken up with two genotypes of pigeonpeas and sorghum CO 22 as intercrop.

Materials and methods

The field experiment was conducted at Tamil Nadu Agricultural University, Coimbatore, during the kharif season, 1984, to study the effect of plant population and intercropping of sorghum CO 22 in two pigeonpea genotypes, namely SA 1 and CO 5. Pigeonpea SA 1 at 40,000 (P_1), 60,000 (P_2) and 80,000 (P_3) plants ha^{-1} and CO 5 at 74,000 (P_1), 1,11,000 (P_2) and 1,48,000 (P_3) plants ha^{-1} were raised as sole and in combination with sorghum CO 22 at 1,48,000 plants ha^{-1} under paired row system of planting (30/90 cm and 30/60 cm respectively). Two rows of sorghum with pigeonpea SA 1 and one row with CO 5 were raised. Spacing between plants was adjusted to accommodate different population levels. Sorghum CO 22, pigeonpea and SA 1 were harvested respectively at 90, 120 and 195 days after sowing. The soil of the experimental field was well-drained sandy clay loam, low in available nitrogen, medium in available phosphorus and high in available potassium. The data were analysed by using factorial randomised block design.

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Results and discussion

Nitrogen uptake

Nitrogen uptake by SA 1 increased significantly with increased plant population from 40,000 to 60,000 plants ha^{-1} at maturity (Table 1). At 80,000 plants ha^{-1} nitrogen uptake decreased due to severe competition among

Table 1

Nitrogen uptake by pigeonpea SA 1 and CO 5 at maturity

	SA 1			CO 5		
	Sole crop	Inter crop	Mean	Sole crop	Inter crop	Mean
P_1	154.24	150.22	152.23	121.24	105.57	113.42
P_2	168.58	163.24	165.91	136.09	122.19	129.14
P_3	161.27	152.95	157.11	143.33	131.70	137.52
Mean	161.37	155.47	—	133.55	119.22	—
CD (P = 0.05)						
System of cropping				1.68	1.15	
Population				2.06	1.40	
Interaction				NS	1.98	

plants. In contrast to this, CO 5 recorded higher nitrogen uptake with increased plant population from 74,000 to 1,48,000 plants ha^{-1}. Intercropping of sorghum CO 22 significantly reduced the nitrogen uptake by pigeonpea plants. Rathnakumar (1983) concluded that among different intercrops, sorghum significantly reduced the nitrogen, phosphorus and potassium uptake by pigeonpea. The magnitude of difference between intercropped and pure cropped plants was much less in SA 1 than in CO 5. This might be due to compensated growth of SA 1 after sorghum harvest in the intercropped situation, because of its longer duration. In CO 5, the extent of reduction was reduced by increasing the plant population per unit area.

Phosphorus uptake

In pigeonpea CO 5 the highest uptake of phosphorus at harvest was recorded by the population of 60,000 plants ha^{-1}, followed by 40,000 and 80,000 plants ha^{-1} (Table 2). Competition among plants at higher density probably caused the reduction in phosphorus uptake. The increased population of pigeonpea CO 5 resulted in higher phosphorus uptake. Intercropping of sorghum CO 22 significantly reduced the phosphorus uptake in CO 5 but little difference was observed in SA 1. This might be due to enhanced dry matter production of intercropped SA 1 after sorghum harvest.

Table 2

Phosphorus uptake by pigeonpea SA 1 and CO 5 at maturity

	SA 1			CO 5		
	Sole crop	Inter crop	Mean	Sole crop	Inter crop	Mean
P_1	12.09	11.91	12.00	8.50	8.03	8.30
P_2	13.04	12.90	12.97	9.81	8.66	9.24
P_3	10.68	10.87	10.78	10.86	9.55	10.20
Mean	11.94	11.89	—	9.75	8.75	—
CD (P = 0.05)						
System of cropping				NS	0.70	
Population				0.25	0.86	
Interaction				NS	NS	

Potassium uptake

As in the case of phosphorus, in pigeonpea CO 5 the maximum uptake of potassium was noticed at the population level of 60,000 plants ha^{-1} at maturity, followed by 40,000 and 80,000 plants ha^{-1} (Table 3). Due to increased competition among plants at a higher plant population, there was

Table 3

Potassium uptake by pigeonpea SA 1 and CO 5 at maturity

	SA 1			CO 5		
	Sole crop	Inter crop	Mean	Sole crop	Inter crop	Mean
P_1	139.44	136.49	137.96	111.41	82.48	101.95
P_2	151.17	143.85	147.51	129.81	105.67	117.74
P_3	135.81	132.16	133.99	134.70	113.94	124.32
Mean	142.14	137.50	—	125.31	104.03	—
CD (P = 0.05)						
System of cropping				2.24	1.81	
Population				2.74	2.22	
Interaction				NS	NS	

a lower uptake of potassium. Whereas in CO 5, potassium uptake increased with increased plant population per unit area. Significant reduction was observed in both the pigeonpea genotypes due to intercropping of sorghum CO 22. The magnitude of reduction was comparatively higher in pigeonpea CO 5 than in SA 1. This might be due to lower dry matter production of

pigeonpea CO 5 in intercropping situation. Soundararajan (1978) observed a significant reduction in potassium uptake by pigeonpea when intercropped with sorghum.

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EFFECT OF PLANTING METHOD, IRRIGATION AND NITROGEN FERTILIZER APPLICATION ON GRAIN YIELD AND YIELD COMPONENTS OF CHICK-PEA *(CICER ARIETINUM)* IN SHENDI AREA, SUDAN

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The experiments were conducted in Shendi area in the Northern Region of Sudan in 1982/83, 1983/84 and 1984/85 seasons. Irrigation interval significantly affected grain yield. The frequent irrigation was better than prolonged irrigation interval during the three seasons. Flat planting gave significantly higher grain yield than ridge planting in two seasons. Application of 90 or 180 Kg N/ha did not affect grain yield and yield components. The increase in grain yield due to frequent irrigation and flat planting were due to production of more pods and seends per plant.

Keywords: Chickpea NEC 2486, yield analysis, irrigation frequency.

Introduction

In Sudan chickpea *(cicer arietinum)* locally known as kabkabie is grown as a winter crop solely in the Northern Region because of suitable climatic conditions. Shendi, where this study was conducted lies in the Northern Region between latitude 16°26'E and longitude 33°26'E. The land races grown are mixtures of kabuli and dehsi types. The seeds are small, sometimes wrinkled, of brown or yellowish colour. The seed is used in various forms for human consumption. The main uses are as balila for ramadan (fasting month) breakfast and as tamia.

No precise estimate of the acreage and production of chickpea is available. Ageeb (1976) estimated the area as 5000 ha with production 0.3—0.5 tons/ha. Yield is low because of low standards of crop management, due to lack of basic agronomic information. Until now little research has been done on the crop. The optimum sowing date is between the end of October and the end of November (Ageeb, 1976). Irrigation is an important cultural aspect because chickpea production in Sudan of the lack of rain in production areas. Other cultural practices such as method of planting and use of chemical fertilizers also need to be investigated.

This study was carried out to determine the effect of irrigation, method of planting and application of nitrogen fertilizer on grain yield and yield components of chickpea.

Materials and methods

Trials were conducted at Shendi Research Farm for three consecutive seasons in 1982/83, 1983/84 and 1984/85. The soil was alkaline, non-saline and non-sodic belonging to the clay textural class with physical and chemical properties of pH, 7.9; ECmmho/cm, 0.6; ESP, 2.7; % total sand, 14; % silt, 36; % clay, 50; total nitrogen, ppm 673; % organic carbon, 0.709; available P ppm 1.4 and available K, meq/100 g 1.54 (Nourai et al. 1984). The mean monthly maximum and minimum temperature for the three seasons is shown in Table 1. The variety used was kabuli type chickpea NEC 2486 which had large size seeds of a beige colour.

Table 1

Monthly mean maximum and minimum temperature at Shendi

Year / Month	1982		1983		1984	
	Max.	Min.	Max.	Min.	Max.	Min.
January	31.4	17.1	25.5	11.1	29.6	14.0
February	29.4	13.6	30.9	14.7	34.0	17.7
March	35.0	19.2	33.3	15.6	38.4	20.9
April	39.9	22.7	38.4	20.9	40.5	22.3
May	41.2	25.9	42.3	26.7	42.7	26.6
June	41.9	28.1	41.8	28.6	42.2	26.9
July	40.7	28.2	41.5	28.4	42.0	26.9
August	39.3	27.6	40.5	27.7	42.2	26.1
September	41.1	28.3	41.5	27.3	42.5	26.8
October	39.0	25.8	39.6	24.1	41.6	25.2
November	32.8	18.1	36.0	20.1	35.5	19.1
December	30.7	14.3	32.0	17.0	31.5	15.7

The treatments consisted of 3 watering intervals (7, 14 and 21 days), 2 planting methods (ridge vs flat) and 3 rates of nitrogenous fertilizer (0 Kg N/ha, 90 Kg N/ha and 180 Kg N/ha) in split plot design replicated 4 times. The irrigation interval was in the main plots, the method of planting in the subplots and the N-fertilizer rate in the sub-sub. plot.

Seeds were sown in plots on either 60 cm ridges or flat in rows 30 cm apart at 20 cm intra-row spacing and 2 seeds per hill. The plot size was 29.4 m^2 of which 14.0 m^2 was harvested for determination of grain yield. At harvest ten plants were taken at random from each plot to determine yield components.

The volume of irrigation water supplied at various intervals for each plot of 29.4 m^2 was 37 m^3, 20 m^3 and 15 m^3 for 7, 14 and 21 days respectively. The N-fertilizer in urea form was broadcast and mixed with soil at planting time. Planting was done on December 1st in the three seasons. Insects and diseases were kept at the lowest possible levels. The crop was harvested by cutting the plants just above the soil surface, stacked in heaps and left to dry for about a week. Threshing was done by beating dry plants with sticks and then winnowed to separate the seeds and the straw.

Results

Grain yield

The effect of main factors (irrigation interval, method of planting and N-fertilizer rate) and treatment combinations on grain yield is shown in

Tables 2 and 3. The irrigation interval significantly ($P = 0.001$) affected grain yield in the three seasons. The highest and lowest yields (1709 and 634 Kg/ha) were obtained from 7-day and 21-day intervals, respectively, with 14 intermediate days. The 7-day interval out-yielded the 14-day by 511 (40%), 794 (61%), 978 (369%) Kg/ha and the 21-day by 936 (111%), 1202 (133%), 1085 (687%) Kg/ha in the 82/83, 83/84 and 84/85 seasons, respectively. The planting method significantly affected grain yield in the 83/84 and 84/85 but not in the 82/83 season. Flat planting increased yield by 98, 305 and 159 Kg/ha over ridge planting in the 82/83, 83/84 and 84/85 seasons respectively. The effect of nitrogen application on grain yield was insignificant but nitrogen application, at various treatment combinations of method of planting and irrigation frequency on grain yield, showed a decreasing trend at short

Table 2

Effec of irrigation interval, planting method and N-fertilizer level on chickpeas grain yield in 1982/83, 1983/84 and 1984/85 seasons Grain Yield Kg/ha

Irrgation interval	1982/83	1983/84	1984/85	Average
7-days	1778	2105	1243	1709
14-days—	1267	1310	265	947
21-days	842	902	158	634
S.E ±	67	74	54	
Sig. level	***	***	***	
Planting method				
Ridge	1246	1286	476	1003
Flat	1344	1591	635	1190
S.E ±	55	60	44	
Sig. level	N.S.	***	*	
N-fertilizer/ha				
0 Kg N	1345	1427	530	1101
90 Kg N	1290	1338	582	1070
180 Kg N	1248	1546	570.0	1121
S.E ±	67	74	54	
Sig. level	N.S.	N.S.	N.S.	

watering intervals (7-day) and an increasing trend at long intervals (21-day). The combination of one week interval, flat planting and 0 Kg N produced the highest grain yield.

Yield components

The effect of treatment factors on yield components is shown in Table 4.

No. of pods/plant. Irrigation interval had a highly significant effect on the number of pods per plant ($P = 0.001$) in the three seasons. The maximum

Table 3

Effect of treatment combinations on chickpea grain yield (kg/ha) *for three seasons*

Treatment* combinations	Nitrogen level (kg/ha)			
	0 Kg N	90 kg N	180 kg N	Average
1WR	1603.6	1531.8	1560.5	1563
2WR	723.9	952.4	785.3	887
3WR	458.1	540.3	679.0	559
Average	995.1	1008.2	1008.3	1003
1WKF	1900.8	1775.3	1897.8	1858
2WKF	1088.0	915.6	1024.9	1010
3WKF	625.9	713.5	787.2	709
Average	1204.9	1134.8	1236.6	1192
R-F	1100.0	1072	1122	

* W = Week
R = Ridge
F = Flat
R.F = Ridge-Flat

Table 4

Effect of irrigation, planting method and N-fertilizer level on chickpea yield components in 1982/83, 83/84 *and* 84/85 *seasons*

Treatment	Pods plant			Seeds/Plant			1000-seed wt.			Plant stand/m²		
	82/83	83/84	84/85	82/83	83/84	84/85	82/83	83/84	84/85	82/83	83/84	84/85*
Irrigation												
7-days	29.1	25.5	19.3	31.9	33.8	30.7	164	174	175	20.1	22.6	36.9
14-days	22.7	18.2	12.1	22.3	23.3	13.6	190	186	178	19.8	21.9	22.9
21-days	23.1	18.4	11.2	16.9	20.7	11.6	189	178	161	20.5	20.8	26.3
S.E ±	1.5	1.2	0.63	1.9	1.7	0.9	4.4	4.7	3.4	0.82	0.7	1.5
Sig. level	***	***	***	***	***	***	***	N.S.	**	N.S.	N.S.	***
N-Fertilizer												
0N	28.7	19.4	14.6	23.8	23.7	17.8	182	179	166	19.9	20.9	27.3
2N	27.0	20.5	13.7	22.0	26.7	18.0	177	181	178	20.7	22.4	28.7
4N	29.2	22.2	14.2	25.4	27.4	20.0	183	179	170	19.7	22.1	30.2
S.E ±	1.5	1.2	0.63	1.9	1.7	0.9	4.4	4.4	3.4	0.82	0.7	1.5
Sig. level	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Planting Method												
Ridge	29.6	19.4	12.1	24.3	23.5	18.4	188	184	171	19.7	21.8	27.6
Flat	27.0	22.0	12.2	23.2	28.4	18.9	190	175	171	20.6	21.8	29.8
S.E ±	1.2	0.96	0.5	1.6	1.4	0.7	3.6	3.9	2.8	0.67	0.6	1.2
Sig. level	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

* Plant stand per unit area

number of pods per plant was produced by the 7-day interval. The rate of N-fertilizer and planting method did not significantly affect the number of pods per plant but, in 83/84, flat planting produced more pods per plant than ridge planting.

No. of seeds/plant. The seeds/plant were significantly ($P = 0.001$) affected by the irrigation interval. The maximum number of seeds/plant were produced by the 7-day interval and then decreased with prolonged irrigation interval. The rate of nitrogen fertilizer and planting method did not significantly affect the number of seeds per plant but, in the 83/84 season, flat planting produced more seeds/plant than ridge planting.

1000 Seedweight Prolonged irrigation intervals produced singnificantly ($P = 0.001$) heavier seeds than did short intervals in 1982/83, but vice versa in 1984/85, with an insignificant effect in the 1983/84 season. The effect of nitrogen application and planting method on seed weight was also insignificant. Plant stand/unit area. The plant stand at harvest was significant in the 84/85 season only, with the 7-day inverval producing more plants than either the 14- or 21-day interval ($P = 0.001$).

The correlations between grain yield and yield components are given in Table 5. Grain yield was positively correlated with the number of seeds

Table 5

Correlation coefficients between and among yield and yield components

	No. seeds/plant	No. pods/plant	100-seed wt.	Plant stand
Seed yield	0.94**	0.63**	(—) 0.73**	0.62**
Seeds/plant	—	0.73**	(—) 0.70**	0.56*
Pods/plant	—	—	(—) 0.77**	0.55*
Seed wt.	—	—	—	(—) 0.55*

Table 6

Significant interaction of method of planting and N-fertilizer level on 1000-seed weight (gm)
(1984/85 season)

Planting method	N-fertilizer level		
	0N	2N	4N
Ridge	174	177	163
Flat	158	179	177

per plant, pods per plant and plant stand ($P = 0.01$) and negatively correlated with seed weight ($P = 0.01$). The seeds per plant, pods per plant and plant stand were positively correlated and negatively correlated with seed weight.

The treatment interactions on grain yield and yield components were insignificant except for the interaction of planting method X Nitrogen fertilizer on 1000-seed weight in the 84/85 season (Table 6). The seed weight decreased with flat planting at 0 Kg N and with ridge planting at 180 Kg N/ha.

Discussion

The irrigation interval was the most important factor in determining grain yield of chickpea. This is of particular importance since chickpea is grown by irrigation because of the arid areas of production. Frequent irrigation produced a consistently higher grain yield in the three seasons than did prolonged irrigation. Saxena (1984) found that frequent irrigation doubled yield in areas of high evaporation. Ageeb (1974, 1975) reported that increasing the watering interval between 7 and 21 days at Hudeiba Research farm had no significant or appreciable effect on grain yield. This indicates the importance of the conditions and the type of soil under which the experiment was conducted. Grain yield increase due to frequent irrigation was attributed to significant increases in the number of pods and seeds per plant. Joshi (1972) reported that the ratio of pods per plant was the most important component contributing to grain yield of chickpeas.

Most of the chickpea acreage in Sudan is in the flooded banks and basins along the river Nile. Farmers usually plant chickpeas on flat areas when the water has receded. Flat planting in this study gave significantly higher grain yield than ridge planting at various watering intervals. This might be due to the better distribution of water in the flat and the relatively available soil moisture in the vicinity of emerging seeds establishing an earlier ground cover for a longer time, resulting in a greater amount of solar radiation intercepted, with increased photosynthesis and a consequently higher grain yield.

Various levels of nitrogen did not improve grain yield. Ageeb (1974, 1975) at Hudeiba Research Station found significant yield increases from application of nitrogen. Ageeb attributed this to the poor status of nitrogen in the soil and perhaps to the absence of the right rhizobium strain for chickpea in the farm soil. In this study the lack of yield improvement due to nitrogen pplication was probably due to efficient rhizobia in the soil that fix nitrogen. in the nodules, which is then transported to the various parts of the plant. Singh et al. (1982) attributed the lack of nitrogen response to the sufficiently high population of native rhizobia for effective nodulation. The response to nitrogen application with a prolonged watering interval application with a prolonged watering interval was probably due to the reduced nitrogen-fixing capacity of the crop. Habish and Mahdi (1976) found that irrigation every 7 days produced better nodulation than every 14 days, due to the dry conditions which prevailed towards the end of the long watering interval.

In conclusion, the results clearly indicated that grain yield was drastically reduced by prolonged irrigation interval due to production of less pods and seeds per plant. The flat planting farmer's method was preferred to ridge planting. The application of 90 or 180 Kg N/ha did not increase grain yield and yield components in comparison with 0 Kg N.

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Plant genetics and breeding

TRYPSIN INHIBITOR CONTENT IN DIFFERENT VARIETIES AND MUTANTS OF SOYBEAN

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Increasing opportunities of utilization of soybean in numerous fields and the emerging shortage of protein in the world require the development of different protein sources.

We must pay attention to the qualitative improvement of crop indices besides increasing the average yield. Nowadays the largest problem of utilizing soybeans is the presence of antinutritive factors in the seed (Kovács 1978), particularly the group of trypsin inhibitors.

If a given low value of trypsin inhibitor (TI) content could be successfully attained by different selection processes, the direct practical importance would be tremendous for improving the quality of basic breeding material. The qualitative changes in protein content caused by heat treatment should be preventable in this way. However, it would indirectly reduce the costs of soybean utilization.

Our aim was the quantitative detection of antinutritive factors in 20 soybean lines and cultivars, as well as the preliminary selection of varieties or mutants which are worth further selecting in order to reduce TI content.

Keywords: Glycine max L., antinutritive factors, trypsin inhibitors, quantitative detection, preliminary selection

Introduction

The most important property of trypsin inhibitor (TI) is its stability against proteolytic decomposition. This considerable stability allows TI to go through the intestines of animals without any changes and inactivation of the present trypsin and, consequently, the protein decomposing processes are inhibited. Thus, the efficiency of consumed protein deteriorates (Kralovánszky 1971). These materials may cause depression in growth, hypertrophy of the thyroid gland and the pancreas and inhibition of important physiological functions.

Many experiments were made in regard to the detection of trypsin inhibitors. As a result of these experiments two main groups of soybean trypsin inhibitors have been found on the basis of their physical, chemical properties as well as the synthesis and degradation in the plant (Stahlhut and Hymowitz 1983).

The Kunitz Soybean Trypsin Inhibitors (KSTI) belong to the first group which are characterized by high molecular weight (21.000), and are

sensitive both to temperature and acid. They can be mainly found in dry seeds and they occur only in soybean. They were named after the researcher who first isolated them in crystalline form.

The inhibitors of low molecular weight (8.000) — called Bowman-Birk Soybean Trypsin Inhibitors (BBSTI) — belong to the second group. They can be found also in leguminous plants. The Bowman-Birk inhibitor itself is a double-headed proteinase inhibitor, with two independent inhibitory sites: one against trypsin and one against α-chymotrypsin.

Inhibition properties of BBSTI are more stable than KSTI's in respect to resistance for temperature, acidic and alkalic agents. Neither papain no pepsin can reduce their activity. Many methods have been devised to detect TI. Singh et al. (1969) used disc-electrophoresis, Hwang (1977) anion change chromatography and Ikenaka (1978) cation change chromatography.

Hymowitz—Hadley (1972) examined two soybean varieties by polyacrylamide gel electrophoresis. In the Harosoy line he found a fast electrophoretic band whose Rf value was 0.79. On the other hand, a slow electrophoretic band occurred in the T-245 variety with Rf 0.75. Both proteins were SBTI-A_2-type trypsin inhibitors. Hill and Breidenbach (1974) separated 3 different types of TI having 2.2 S, 7.5 S and 11.8 S sedimentation coefficients. Most of the trypsin inhibitors located in mature soybean seed had a value of 2.2 S.

Trypsin inhibitors can be isolated by different methods, such as DEAE-cellulose chromatography (Rackis et al. 1962, Tan-Wilson et al. 1982) and by gel filtration on Sephadex column (Tetsujiro and Miyo, 1967, Ellenrieder et al. 1980).

Stahlhut and Hymowitz (1983) isolated the BBST-5 type by anion exchange column chromatography in Amsoy-71 line.

Proteinase-inhibitors form a special group of plant proteins. The trypsin inhibitor content in soybean seed is about 6% of average protein content. There are significant differences in the occurrence of TI of low and high molecular weights.

Both types of TI were found in the majority of varieties (71%) examined by Stahlhut and Hymowitz (1983) and only 3% of seeds contained no TI of low molecular weight. High genetic variations in soybean varieties probably appear not only in qualitive but also in quantitive properties of TI.

Starting out from this conception, the TI level of seeds of several soybean cultivars and mutants was examined, taking into consideration that favourable types can be applicable in breeding work.

Materials and methods

Eight soybean varieties and twelve mutants were examined (Table 1). Mutants derived from a selection of mutant population, produced by ^{60}Co gamma irradation in the gamma field of Department of Genetics and Plant Breeding. Average samples were collected in

1985 from the Hatvan-Nagytelek Experimental Station of University of Agricultural Sciences, Gödöllő, Department of Genetics and Plant Breeding. The determination of trypsin inhibitor content in uncooked soybean seed was made by the Laboratory of Hidasháti State Farm. The method employed by us is the same as the method of agricultural and foodstuff standard (number: MSZ-08 1833—83). Measurements were repeated three times.

Granules made from uncooked soybean seed were defatted using a classical method (with petrol-ether in Soxhlet apparatus) and ground to 0.1 mm granules. After shaking the soybean flour in aqueous suspension for 3 hours and adjusting the pH to 9.5—9.8, a reagent was added: N-αbenzoyl-DL-arginine-p-nitroanilide-hydrochloride (DL-BAPA, Merck) at 37 °C. In addition to artificial substrate (DL-BAPA), exogen bovine trypsin was also used. Absorbance of p-nitroaniline released during the reaction was measured by spectrophotometer at 410 nm wave-length, 30 minutes after stopping the reaction. There was a linear relationship between released p-nitroaniline and enzyme-activity.

One trypsin inhibitor unit (TU) is that activity which decreased the trypsin activity by one unit — under standard conditions — and is equal to one trypsin inhibitor international unit (TIU).

Table 1

Results of trypsin activity measurement

Number	Genotype	Trypsin inhibitor international unit (TIU/mg defatted soybean flour)	TIU/mg defatted soybean flour expressed in the % of ISz-15
1.	ISz-15	215.6	100.0
2.	Semundo-20	191.2	88.7
3.	"A" mutans	171.6	79.6
4.	BS-31	163.2	75.7
5.	511	79.6	36.9
6.	993	78.4	36.4
7.	842	68.0	31.5
8.	S 1346	67.2	31.2
9.	893	58.8	27.2
10.	784	52.4	24.3
11.	65	52.0	24.1
12.	693	50.5	23.4
13.	Kárpátalja	48.8	22.6
14.	BS-33	48.0	22.8
15.	773	47.6	22.1
16.	765	38.4	17.8
17.	"B" mutans	32.8	15.2
18.	994	32.0	14.8
19.	841	30.8	14.3
20.	891	20.8	9.6
L.S.D.5%		20.04	

Results and discussion

Soybean genotypes can be classified with high accuracy by means of the trypsin inhibitory level measurement. Cultivar ISZ-15 contained the highest amount of antinutritive factors —215.6 TIU/mg defatted soybean seed (Table 1).

Measurement of the seed material, derived from the same morphology and age-group, calls attention to important differences in the amount of antinutritive factors among certain varieties in uncooked soybean seed (48.0—

215.6). It would be worth noting this fact during the utilization of soybean seed (both for feeding and food industry) when the period of heat treatment for inactivation is determined (30—40 minutes at 102—103 °C in practice). Thus, not only the energy requirement could be decreased, but also more important, the injury to biologically indispensable valuable protein components caused by high temperature minimized. Products are applicable utilization in the food industry when their trypsin inhibitor content is under 20 TIU/mg (Bátkai, J. 1987). For this reason, mutant 891 which approximate this limit has a striking importance. The same good results (about 30 TIU/mg) were provided the genotypes 994, B and 765.

The well-known variety Isz-15 can be considered a standard. In comparison with this standard, all genotypes differ from it significantly (Table 1).

In conclusion, there is a real opportunity for reducing the trypsin inhibitor level in soybean genotypes, using genetic and plant breeding methods, to such a low amount that the uncooked soybean seed would be directly available in feeding after a considerably shorter time of heat treatment, or without heating.

Summary

Eight soybean varieties and twelve mutants were tested for trypsin inhibitor activity using the MSZ-08 1833—83 standard. Significant differences were found in amounts of trypsin inhibitors containing uncooked soybean seed (48.0—215.6 TIU/mg defatted soybean flour). It seems practical to note this fact during the utilization of soybean seed (both for feeding and food industry) when the period of heat treatment for inactivation of antinutritive factors are determined.

Some genotypes were found among the mutant lines in which the TI content was so low in uncooked soybean seed that there were no significant differences from the critical value desirable in the food industry.

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MORPHOGENETIC FEATURES OF SEED-PRODUCING STONE FRUIT ROOTSTOCK VARIETIES

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In 5-year periods of examination between 1976 and 1984 the author collected flowers from virus-free trees of 6 almond-, 2 *Prunus amygdalopersica*-, 2 wild peach, 17 *Prunus armeniaca*-, 10 plum, 2 wild sweet cherry- and 4 *Prunus mahaleb* rootstock varieties. Measurements were taken of the petiole length, petal median, length of filaments on the outer androecium, pistil length, stigma diameter, pollen size, pollen germination; also the stigma/pollen ratio, the relative stamen number and the frequency of defective flowers were determined; other characters (15) since they did not prove specific of variety are only mentioned in the section Materials and Methods; data on autogamy are completed with the results of investigations by Erdős (1974).

The morphogenetic components of self-fertility are: petal median (mm), stigma diameter (μm), relative stamen number (n/mm) and apistilly (%). There is a very close correlation between the major characters; for the morphological possibility of fertility particularly important are the well-developed pistil, the inclination to apistilly, the petal median (insect pollination) and the pollen germination as well as their correlations.

The phenotypic effect is extremely strong by autogamy, pollen germination and apistilly; otherwise the varietal effect is 2.17 times stronger (on the basis of CV, %) than the year effect. Yet, the role of years is to be taken into account, since it expresses the sum of the ecological effects in the period of flower bud formation. In rainy years, flowers with longer pistils develop, and the organization disorders of the gynoecium also increase if the rainy weather is combined with low temperatures in the first half of the period of flower bud formation; dry weather rather favours the stamen formation.

The present study supplied new data for the description and identification ol varieties by flower, and contributed to the conditions of fertilization (morphologicaf and ecological components); the investigations will be extended to cover stone fruit rootstock varieties of full bearing age.

Keywords: almond, amygdalopersica, bird cherry, bullace, ecological effects on flower, flower differentiation, mahaleb, myrobalan, sex expression, wild apricot, wild peach wild sweet cherry.

Introduction

Prunoideae is a very important subfamily of the family *Rosaceae*, as all cultivated stone fruit scion- and stock species belong here. Important root. stock species of Hungary are: bitter almond *(Amygdalus communis* L. convar. *microcarpa* provar. *amara)* and sweet almond *(Amygdalus communis* L. convar. *microcarpa* provar. *microcarpa)*, *Prunus amygdalopersica* (*Amygdalo-*

persica×hybrida Soó), wild peach *(Persica vulgaris* Mill. convar. *persica* provar. *persica)* and nectarine (*Persica vulgaris* Mill. convar. *laevis* provar. (*scleronucipersica* (cling-stone)).

The wild apricots belong to the *Armeniaea vulgaris* Lam. convar *minor* small-fruited) and convar. *vulgaris* (large-fruited) taxon, the bullace (*Prunus institita* Jusl.) and the myrobalans *(Prunus cerasifera* ssp. *divaricata* and sp. *pontica)* also are used as rootstocks.

In a botanical sense the wild cherries belong to *Cerasus avium* Mönch. ssp. *avium* forms, and the mahalebs can be placed in the *Cerasus mahaleb* Mill. ssp. *Simonkaii* group. The description of taxa has been carried out by Soó (1966), Terpó (1984) and Surányi (1979) etc. However, systematic studies on fertilization and flower morphology have hardly been made so far; the only exceptions in a certain sense are the studies by Erdős (1984) and Surányi (1979, 1980b, 1985).

Nyújtó and his colleagues collected local varieties from the fifties, then made selection work. The virus-free seed-producing stock plantation at Cegléd is the most important result of this work (Nyújtó, 1987). This because of the small number of data on flower biology the importance of the present study is notable.

On the fertility conditions of seed-producing stone fruit rootstock varieties in Hungary, we only possess a few "indirect" data (Surányi, 1979 and 1980b), and have some concrete data on autogamy and geitonogamy (Erdős, 1984). Almond generally is a self-sterile species, the S_1S_2 alleles induce self-sterility, though several exceptions are known; e.g. José Dias and Duro Italiano (Almeida 1945) are self-fertile, and Non Plus Ultra when associated with an optimum pollen partner may give some 30% fruit setting. Abnormities of the flower (mainly of the pistil and ovary) and a poor viability of pollen most affect the fertility; sterility is the consequence of multiallele effects, the pollen tube cannot develop in the stigma tissue of the same plant.

As for the peach varieties, we know more about the genotypic determination; the d_1d_1, dm_1 and dm_2 allele pairs bring about types with very large petals; for pollen sterility the ps allele pair is responsible (Connors 1928, Scott and Weinberger 1944, Lammerts 1945). Self-fertility is a feature of dominant inheritance. The *Persica vulgaris*×*Amygdalus communis* hybrids are, therefore, much more inclined to self-fertility than the almond varieties and forms; it is due to this characteristic and their higher frost tolerance, compared to the almond, that these hybrids are much favoured in nurseries.

The wild apricots show a diversified picture: there are small- and large-fruited, sweet- and bitter stoned, frost tolerant and frost sensitive types. The varieties also differ in inclination to self-fertility; at least 15—25% of the varieties examined are geitonogamous, while numerous forms and clones were found in our experiments to demand cross-pollination.

The wild cherries are self-sterile in Hungary almost without exception (Erdős 1984), but the southern ecotypes in the Mediterranean region and at the foot of the Caucasus can even be self-fertile (Tóth and Surányi 1980). The bullace is, on the other hand, self-fertile, though the world assortment shows a diversified picture. The stamen number is genetically controlled for all stone fruit species and -varieties (Haskell 1954, Haskell and Dow 1955, Morrison 1964, Surányi 1974); 15—33 stamina are in the flowers, though in extreme cases under certain conditions some varieties may be exceptions (Surányi 1985).

The cultivated cherries are mostly self-sterile (Crane and Lawrence 1952), the S_1—S_9 alleles cause incompatibility (Anonymus 1963): there is no pollen in the anthers, or besides the absence of pollen there is not stigma activity either, which is due to the S_1S_1 allele pair. According to Funk (1958) 5% of the mahaleb trees are self-fertile, so also in Hungary the local variety Korponay V. (Sebők 1968); as a matter of fact, similar observations were made by Joley (1943).

To summarize, with a view to seed production by good rootstock varieties, it is fundamentally important to know their inclination to self-fertility, which — since they are wild forms — can be modified by cultivation. The results to be found in works by Hedrick et al. (1911), Taylor (1949), Crane and Lawrence (1952), Soó (1966) and Nyéki (1980) have so far been the most important of all. Full information on the origin and hybridization of *Prunus* species can be found in Rybin (1936), Kárpáti (1967), Soó (1966), Terpó (1974) and Salasses (1975). Here we only refer to these basic works.

Materials and methods

The author has been dealing since 1968 with questions of flower organization in stone fruits, partly with the functional and corrective factors of the flower parts, partly with a pomological description of flowers (serving the description and identification of varieties) and their climatic dependence. The bases laid for the present study can be read in a number of papers (Surányi 1970, 1972, 1979, 1980b, 1983, 1985).

The periods of the examinations and the varieties included in them (SF = self-fertile, PSF = partially self-fertile, PSS = practically self-sterile, SS = self-sterile) are seen below (Surányi 1985):

	SF	PSF	PSS	SS	Total
1976—1980 almond	—	—	2	4	6
P. amygdalopersica	—	2	—	—	2
P. mahaleb	—	—	3	1	4
1977—1981 wild peach	2	—	—	—	2
1979—1983 wild cherry	—	—	1	1	2
1980—1984 wild apricot	7	9	1	—	17
bullace	1	—	7	2	10
Total	10	11	14	8	43

Rootstock varieties of stock orchards registered at Cegléd are marked "C", but the serial number is accidental and does not refer to the time of collecting. According to the fertility groups set up by Tóth (1967) the varieties were as follows:

- almond: C.431 and C.472 practically self-sterile; C.446, C.447, C.449 and C.471 self-sterile;
- *P. amygdalopersica:* C.410 and C.465 partially self-fertile;
- wild peach: C.932 and C.2629 self-fertile;
- wild apricot: C.809, C.1301, C.1426, C.1650, C.1652, C.30235 and C.31625 self-fertile; C.145, C.195, C.303, C.615, C. 694, C.1300, C.1620, C. 1879 and C.2546 partially self-fertile; C.155 practically self-sterile;
- bullace: C.83 self-fertile; C.162/a, C.162/b, C.174, C.359, C.679, C.801 and 1/15 practically self-sterile; C.364 and C.767 self-sterile;
- wild cherry: C.2493 practically self-sterile; Altenweddingeni self-sterile;
- *P. mahaleb:* C.500, C.2753 and SL 64 practically self-sterile; Érdi V. self-sterile (Altenweddingeni and SL 64 are of foreign origin).

The flowers were collected at the beginning of opening from virus-free trees of each variety, mostly from spurs. Measuring and examination were carried out in the following way. For each flower we took the weight, the petiole length (except for almond-, amygdalopersica-, wild peach- and wild apricot varieties), the pistil length and the stigma diameter; counted the fertile and sterile stamina in the flowers ($n = 30$). For every third flower ($n = 10$) we measured the length of the calyx tube, the length and width of sepals and petals and the length of filaments on the outer and inner circle of stamina.

In addition we determined the size of anther and pollen in every ninth flower ($n = 4$) of each of the 43 varieties. Pollen germination took place in laboratory, in 10% saccharose, in 24 hours of incubation at 21 °C. The flowers were weighed by an assay balance, the size of the flower parts was measured with millimetre accuracy. The size of stigma and anther was determined by a binocular microscope at 25× magnification. The size of the pollen and the rate of tube development were determined at 640× magnification under a light microscope, similarly with a stage micrometer.

The teratological changes of gynoecium and androecium were expressed in percentage for 30 flowers per variety every year; such abnormities are: acarpellous and polycarpous flowers and transformation of filaments into petals (cf. Surányi 1972, 1985).

The calculated values were obtained from the corresponding data: the relative stamen number, the number of fertile stamina per unit length of pistil; the sepal-, petal-, anther- and pollen shape index (the ratio of length and width), the petal median (the mean value of length and width), the ratio of stigma diameter/pollen size (on the basis of the 1st, 10th, 19th and 28th flower); the percentage frequency of apistilly (acarpelly?), polycarpy and staminody from 30 flowers; pollen germination is also a percentage value (the percentage value of pollens developing tubes 3—4 times the size of pollen compared to the total number of pollens).

Fertility (autogamy) examinations were done simultaneously with the present research programme; at least 200 flowers of each variety were isolated (Erdős 1984).

After the evaluation and analysation of a very large number of data it was found that the petiole length, the petal median, the length of filaments on the outer circle, the pistil length, the stigma diameter, the pollen size, the pollen germination, the stigma/pollen ratio, the stamen number, the relative stamen number and the frequency of apistilly were actually informative concerning the fertility conditions of the varieties.

The significance calculations were made every year for each variety, the dependence on year was analysed on the basis of standard deviation, and for the most important characters analysed on the basis of standard deviation, and for the most important characters also by a comparison with the climatic data of the Cegléd Meteorological Station; in further regression analyses the correlations of the morphogenetic characteristics were examined (Sváb 1981). All data are not published in this paper as a detailed account of the varieties was already presented at an earlier symposium (Surányi 1976).

Results and discussion

The genotypic and phenotypic effects on the characters concerned were analysed by standard deviation; on the basis of autogamy, pollen germination and apistilia variety has the greatest influence, though occasionally the effect

of year may also be considerable. As a further conspicuous phenomenon, the rate of pollen germination is a strong genotypic characteristic only in the almond and amygdalopersica varieties. For the other characters standard deviation by the variety was 2.17 times higher than that by the year (CV, %). On the other hand, the diversity of varieties by relative stamen number was the greatest in almond, wild peach and *Prunus mahaleb*; the pistil length remarkably varied in the amygdalopersica and wild apricot flowers; the petiole lengths of bullaces and wild cherries also show wide fluctuations (Table 1).

In pure plantations a good yield cannot practically be expected from almond trees. In the variety C.447 the petal median is very small and the outer stamina are short, while the pistile are the longest (18.0 mm) in this variety. The pollen grains of the almond varieties C.431 and C.449 develop their tubes in 10% saccharose very poorly and at low intensity. The tube development was but slightly better in the other varieties. This is one of the reasons why *Prunus amygdalopersica* should be taken into consideration as a pollen partner. The stigma diameter and pollen size were hardly dependent on the variety, while as regards the relative stamen number C.449 and C.446 showed wide extremities. Both varieties showed self-sterility of extreme masculine and feminine character, and in C.449 defective flowers frequently occurred.

The small number of *Prunus amygdalopersica*- and wild peach varieties examined (2 of each) is not enough for the purpose of comparison; on the other hand, in comparison with the almond species, the examination of intervals is very instructive. In both varieties the petal median differs from that in almonds, though in opposite directions. The size of pollen exceeds the pollen size of almonds, and the viability of the pollen grains is also such better. The stigma/pollen size ratio exceeds the corresponding value of almonds, and the stamen number and relative stamen number are somewhat different (Table 2).

For three almond varieties (C.431, C.447 and C.471) the data of the generative organs were analysed between 1969 and 1972 (Surányi 1979); the difference is pistil length and relative stamen number was insignificant only in C.447. The variation of pistil length and the ecosensibility of certain varieties are explained partly by the age of the trees (the earlier examinations covered old trees, while the trees in the present study were 8 years old), partly by year differences. For *Prunus amygdalopersica*- and wild peach varieties there are data only from one year each, so it is of no use to compare generative organs of flowers from the old plantations. The right line of selection is indicated by the fact that none of the 8 varieties in Table 1. showed any inclination to apistilia, although Knight (1969) describes the almond as a species inclined to apistilia.

Table 1

Changes in the plant parts and fertility conditions (CV, %)

Examination	Almond		*P. amygdalopersica*		Wild peach	
	Variety	Year	Variety	Year	Variety	Year
Autogamy, %	244.0	70.5	16.3	95.8	31.1	101.8
Petiole length, mm	—	—	—	—	—	—
Petal median, mm	17.3	1.2	2.5	4.3	11.8	2.6
Stamen length (outer), mm	12.1	2.9	13.9	5.5	12.0	1.4
Pistil length, mm	10.1	4.6	19.0	18.1	9.7	8.8
Stigma diameter, μm	12.3	3.0	18.0	9.4	0.3	2.3
Pollen size, μm	11.6	3.0	14.5	3.7	5.6	1.9
Pollen germination, %	74.0	6.2	82.5	70.2	7.4	5.7
Stigma/pollen ratio	11.0	5.5	4.6	13.3	10.0	4.7
Stamen number, n	20.5	2.7	6.9	5.1	3.6	8.8
Relative stamen number, n/mm	22.4	4.8	1.7	10.2	11.9	4.0
Apistilia	150.9	127.6	141.4	91.6	95.4	88.7

Table 2

Morphogenetic characterization of almond, Prunus amygdalopersica and wild peach

Variety	Years-Autogamy % +	Petal mm	-Stigma diameter μm	Pollen germination %	Relative stamen number n/mm	Apistilia %
Almond	1976—1980					
C. 431	0.1	14.0	1147	7.5	1.91	0
C. 472	0	14.0	1392	15.6	1.73	0.3
C. 446	0	13.3	1096	19.5	2.41	1.0
C. 447	0	8.3	1008	17.9	1.61	0.1
C. 449	0	11.6	1194	4.7	1.02	2.5
C. 471	0	15.0	1275	30.4	1.90	0.3
P. amygdalo-persica	1976—1980					
C. 410	9.7	11.2	951	51.4	1.63	0
C. 465	7.7	11.6	1228	13.5	1.67	0.3
SD. 5%	0.92	0.51	78.0	14.27	0.17	—
Wild peach	1977—1981					
C. 2629	19.1	6.6	1172	47.5	2.44	3.6
C. 932	12.2	7.8	1177	43.2	2.06	0.7
SD. 5%	5.94	0.93	34.2	6.55	0.08	2.81

* Data by Erdős (1984)

Table 3 summarizes the extreme morphogenetic characters of wild apricot varieties. We found some exceptional wild apricots: C.1620 has remarkably small petals (8.7 mm), in C.1879 and C.1300 the filaments are very long;

as a function of variety and year

Wild apricot		Plum		Wild cherry		*P. mahaleb*	
Variety	Year	Variety	Year	Variety	Year	Variety	Year
66.1	73.7	18.1	17.2	141.4	346.4	141.4	76.1
—	—	17.4	2.7	14.3	2.7	14.3	2.9
8.9	3.7	13.2	3.9	4.0	1.9	12.3	1.6
12.7	2.2	11.0	2.2	6.6	1.1	14.2	2.2
26.5	7.6	10.5	8.2	4.9	1.1	14.8	16.9
17.4	5.7	8.2	12.9	8.4	4.5	10.1	4.9
21.9	2.1	7.7	12.3	7.2	5.8	2.5	8.5
23.6	19.4	45.9	19.5	9.3	7.2	23.5	5.6
16.0	2.6	11.5	3.7	6.7	5.0	7.2	6.8
25.7	5.4	2.9	1.6	1.1	1.7	4.9	3.5
13.0	6.0	14.5	4.5	7.0	2.7	18.8	16.2
73.2	103.5	78.1	165.6	0.0	223.6	—	—

C.1300 and C.30235 have very large pistils (18.0—18.1 mm) while the flowers of C. 1426 are characterized by relatively short pistils (13.6 mm); the stigma diameter in C.195, C.1426 and C.1650 is below 700 μm, while in C.30235 it comes close to 1200 μm. In pollen size three varieties excelled: C.155, C.1879 and C.30235 have significantly larger pollens (81.9, 76.5 and 72.7 μm, respectively) than the other wild apricots. Relatively poor pollen viability was only observed for C.1426, while the pollens of C.809 and C.1300 gave very high percentages of pollen germination. In C.155 the stamen number exceeds 32, while in C.1650 it is hardly more than 26. The relative stamen number is rather constant in the wild apricots, apistilia only shows a high percentage in C.145 (5.6%) and is coupled with increased frost sensitivity (cf. Erdős 1984) (Table 3).

In the self-sterile C.364 and C.767 myrobalans the petiole, the outer filaments and the pistil are very short; similar correlation was earlier observed in the variety Alutscha (Surányi and Tóth 1976). The differences in stigma diameter are negligible; in C.359 compared to the other varieties the pollen size is outstanding (71.2 μm, C.162/a is a poor while C.767 a very good pollen donor (24.3 and 70.6%, respectively).

The bullace marked C.83 falls far behind the other wild plums as regards the stamen number, and its relative stamen number is similarly. Several myrobalans showed high inclination to forming defective flowers (C.162/b, C.359, C.364 and C.767), they represented 10% of the total number of varieties examined (1983). The preceding period (1982 June-November: from flower bud formation to the beginning of dormancy) was characterized by dry weather.

Table 3

Morphogenetic characterization of wild apricot rootstock varieties

Variety	Years Autogamy % +	Petal median mm	Stigma diameter μm	Pollen germination %	Relative stamen number n/mm	Apistilia %
Wild apricot 1980—1984						
C. 1426	19.6	10.3	666	35.7	2.19	0.6
C. 1301	19.4	10.2	908	68.8	1.73	3.2
C. 1652	15.8	9.2	783	42.3	2.03	1.6
C. 1650	14.9	10.7	686	58.0	1.75	2.6
C. 31625	13.6	10.1	1064	69.3	1.97	2.6
C. 809	13.5	11.3	810	72.4	1.72	0
C. 30235	11.4	11.9	1184	55.5	1.45	0.4
C. 1870	8.9	10.7	1044	47.3	1.93	3.2
C. 1620	8.0	8.7	939	58.3	1.78	3.4
C. 615	7.7	10.7	1006	39.7	1.36	1.0
C. 195	6.9	9.8	665	40.3	1.85	2.4
C. 2546	6.8	12.0	819	45.0	1.68	0.8
C. 694	3.6	9.5	972	46.9	2.04	3.8
C. 303	2.7	10.5	912	40.5	1.65	1.6
C. 145	2.4	11.2	840	65.0	1.91	5.6
C. 1300	2.2	11.8	985	71.1	1.47	0.2
C. 155	0.05	11.3	1135	46.8	2.14	4.4
SD. 5%	6.12	0.31	79.6	9.34	0.13	1.97

* Data by Erdős (1984)

The varieties C.162/b and C.174 (26—30 years old trees) were included in another series of examinations between 1977 and 1979 (Surányi 1980b); the present data call attention to a remarkable difference; namely, in young trees the pistils are very long but the flowers often show abnormities and are incapable of functioning, while trees of full bearing age are much more productive. On the other hand, the mother tree (28 years old) of C.83 and its present progenies (8 years old) hardly differ by flower; this characterizes, otherwise, the ecologically highly stable *P. institia* varieties, as opposed to the high (frost and) ecological sensitivity of the myrobalans. The difference in self-fertility between wild apricots and bullaces is accompanied by differences in filament length, pistil length, pollen germination and the relative stamen number between them (Table 4).

Table 5 contains the morphogenetic characters for the two wild cherry- and four *Prunus mahaleb* varieties. The two species showed differences in petiole length, length of outer filaments, size of pistil, stamen number, and consequently also in the relative number of stamina. In two respects these species were similar: in self-fertility and negligible abnormities of gynoecium.

Further years of observation are required to decide whether in the genus *Cerasus* the autogamy of the first years sufficiently characterizes the inclina-

tion to self-fertility in full bearing age; these two species are hardly getting to become cultivated species within ten years. A comparison of the present data with information on the original habitats shows that the behaviour of

Table 4

Morphogenetic characterization of plum rootstock varieties

Variety	Years Autogamy % +	Petal mm	Stigma diameter μm	Pollen germination %	Relative stamen number n/mm	Apistilia %
Bullace 1980—1984						
C. 83	10.1	9.6	851	71.3	2.64	0
Myrobalan 1980—1084						
C. 162/a	0	7.9	819	24.3	3.80	1.8
C. 679	0.05	8.1	823	32.2	3.84	1.8
C. 162/b	0.05	7.8	778	32.3	3.33	4.0
C. 174	0	6.6	780	47.3	4.20	1.2
C. 359	0	6.0	800	60.9	4.19	4.0
C. 801	0	6.5	803	9.6	3.64	4.6
1/15	0.3	7.7	985	56.9	3.74	0
C. 364	0	5.8	801	44.6	5.36	4.0
C. 767	0	5.2	762	70.6	4.43	0.6
SD. 5%	2.69	0.45	86.7	8.73	0.12	4.94

** Data by Erdős (1984)

Table 5

Morphogenetic characterization of wild cherry and P. mahaleb rootstock varieties

Variety	Years Autogamy % +	Petal mm	Stigma diameter μm	Pollen germination %	Relative stamen number n/mm	Apistilia %
Wild Cherry 1979—1983						
C. 2493	0.2	8.7	999	55.9	2.87	0.04
Althen-weddingeni	0	9.2	1125	49.0	2.60	0.04
SD. 5%	—	0.38	89.5	9.83	0.28	0.25
P. mahaleb 1976—1980						
C. 500	0.2	6.6	888	41.3	2.74	0
C. 2753	0.1	7.1	799	56.5	2.08	0
SL 64	0.1	6.5	986	72.3	2.54	0
Érdi V.	0	8.4	805	50.8	1.79	0
SD. 5%	0.24	1.04	17.8	14.35	0.61	—

* Data by Erdős (1984)

wild cherries and mahalebs in their original habitats differed from their behaviour at Cegléd as grafts: the flowers became more feminine. However, it is not known as yet whether the phenomenon is due to a lasting (ontogenetic) effect or it is the result of an ecological modification.

The climatic dependence of the organization of the generative organs is seen in Table 6. Since we used the June to November data of a five-year period, we did not expect consequent tendencies and correlations. However, the seven stone fruit rootstock species together called attention to interesting correlations; namely, total temperature and apistilia, total amount of precipitation and stamen number and apistilia were found to be in close correlation. Cold, rainy weather in the initial phase of flower bud formation increases the fruit organization disorders many times; although — as it can be seen — great (lasting) anomalies of weather are not favourable for the androecium either (Table 6).

The major morphogenetic characters very often show close correlations, as proved by the regression analyses; the petiole length of short-petioled species was not taken into consideration, being a non-specific character. The correlation between stigma diameter and pollen size was the least demonstrable, and out of the species examined it was for *Prunus amydalopersica* that the fewest proved correlations were obtained. The length of petiole is a suitable indicator of structural (pistil) sterility. Self-fertility cannot be proved with the relative heights of stigma and anthers, since the relation between pistil length and length of filaments follows a positive trend (cf. Kobel 1954).

Highly important is the negative correlation between pistil length petal median and relative stamen number. The size of pistil and the inclination to apistilis can be in negative correlation in so far as the data of defective are left out of consideration, because we found that abnormity of gynoecium was also observed in varieties with extremely large pistils (Table 7).

Considering the fertility groups we do not show the most important morphogenetic characters because of difficulties in grouping them; but even on the basis of varieties not necessarily representing the species, it can be proved that owing to certain rules of organization the inclination to self-fertility is in correlation with petal median, stigma diameter, pollen germination, relative stamen number and apistilia. In the case of feminine species (almond, *Prunus amygdalopersica*, occasionally wild peach) and rootstock varieties of masculine character *(P. myrobalan*, wild cherry, *P. mahaleb)* organization modifications of generative organs occurring for some reason may cause favourable and unfavourable changes alike). All this was presented earlier as a model, and referred to in an important publication (Surányi 1976).

To sum up the results of these 5-year periods of examinations, it would be very important to continue the investigations up to full bearing age, partic-

Table 6

Dependence of pistil length, stamen number and apistilia on total temperatures and amount of precipitation during flower bud formation

Correlations		Almond	*P. amygdalo-persica*	Wild peach	Wild apricot	Plum	Wild cherry	P. mahaleb	Total
Temperature total	pistil length	−0.569	−0.806	−0.037	−0.446	−0.825	+0.056	−0.441	−0.267
	stamen number	+0.153	+0.509	+0.350	−0.559	−0.111	+0.918	+0.422	+0136
	apistilia	−0.047	+0.219	−0.708	−0.732	+0.746	+0.550	***	−0.855***
Precipitation total	pistil length	−0.046	−0.257	−0.481	−0.510	−0.321	−0.330	−0.280	−0.101
	stamen number	−0.584	−0.179	−0.318	+0.568	−0.049	+0.778	−0.795	−0.905***
	apistilia	−0.293	+0.523	−0.495	−0.026	−0.673	−0.084	***	−0.586***
					r 5% = 0.8783				0.3494

*** p = 0.1%

Table 7

Major correlations of the morphogenetic characters

Correlations	Almond	*P. amygdalo-persica*	Wild peach	Wild apricot	Plum	Wild cherry	P. mahaleb	Total
Petiole length and pistil length	***	***	***	****	+0.815	+0.711	+0.953	+0.944***
Pistil length and stamen number	−0.531	−0.687	−0.411	−0.202	−0.898	−0.702	−0.991	−0.404***
Pistil length and filament length	+0.478	+0.574	+0.705	+0.244	+0.978	+0.808	+0.909	+0.952***
Stigma diameter and pollen size	+0.493	+0.388	+0.803	+0.960	+0.145	+0.401	+0.328	+0.371***
Petal median and rel. stamen number	−0.441	−0.212	−0.667	−0.434	−0.664	−0.745	−0.905	−0.698
Pistil length and apistilia	−0.408	−0.604	−0.514	−0.183	−0.408	−0.604	−0.514	−0.183
r 5%***	0.3809	0.6319	0.6319	0.1946	0.3246	0.6319	0.4438	0.1946
r 1%	0.4869	0.7646	0.7646	0.2540	0.4182	0.7646	0.5614	0.2540
r 0.1%	0.5974	0.8721	0.8721	0.3211	0.5189	0.8721	0.6787	0.3211

ularly when for various reasons the trees are cut back every year. Likewise, a study of the effect of cultivation is decidedly interesting both from the point of view of cultivation history and physiomorphology. The varietal specificity of the characters concerned suggests that they are suitable to describe the rootstock varieties more exactly, to distinguish them, and to detect possible cases of crossing. On the basis of the conclusions of such examinations, the methods of evaluating the rootstock varieties can be more accurately determined, and variety combinations for a future stock plantation can be planned.

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THE VARIABILITY OF MALE STERILITY IN THE P-ROGENIES OF INBRED CMS-GENOTYPES

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The six parental cms-genotypes used in the experiment were selected in the field on the basis of above-average open-pollinated fertilization. Analyses were begun on 2×25 progeny of each maternal family. The relation between parental male sterility and progeny characters was studied using the correlation pairs method, and the correlations between individual progeny characters by means of path analysis.

Pollen-sterile individuals were found with high frequency in the open-pollinated progeny of genotypes with a high degree of male sterility. A relatively slight reduction in male sterility was indicative of female (cytoplasm) dominance.

It is very probable that it will prove easier to find a large number of mainteners for male sterile genotypes which produce highly sterile progeny even in the case of open-pollination, thus facilitating more effective selection for other characters.

The results of multiple regression analysis indicate that selection should be fundamentally based on the principal yield components. The slight interaction between seed size and genetic yield potential was insufficient to demonstrate greater vitality in plants raised from larger seeds.

Keywords: Alfalfa hybrids, male sterility, OP-progenies.

Introduction

The majority of new alfalfa varieties are synthetic. Since the 1960s more and more research has been aimed at elaborating new alfalfa breeding methods whereby yield potential can be increased through the exploitation of genetic effects (e.g. interactions between alleles and genes) which have not previously been applied in practical plant breeding. The uncontrollable population changes appearing during the variety maintenance of synthetic varieties also mean that the varieties do not remain in general cultivation for a long time.

Hungary was the first country in Europe to produce KM-Hybridalfa (Lázár 1981) alfalfa, whch has supplied the following important data: 1. The yield potential of combinations produced using specific parents may be higher than that of the best synthetics currently under cultivation. 2. In certain cases it may be possible to achieve genetic complementation in the progeny (the joint appearance in the progeny of earliness and good tillering, which were found separately in the parents). 3. The hybrid vigour may result in

a greater resistance (adaptive heterosis), which is an advantage with respect to both yield potential and persistence in an environment severely infected with *Fusarium* spp., for example.

In addition to these favourable effects, certain disadvantages must also be expected in the course of hybrid production: (1) If a new hybrid is to gain ground, not only the profitability of production is important, but also the seed price level compared to that of synthetic varieties. (2) Hybrids require favourable conditions and their genetic potential can only be transferred into surplus yield if these conditions are fully ensured. Unfavourable changes in environment or technology will lead to yield reductions. (3) In the course of mass vegetative propagation (with a large number of "clone generations") a series of micromutations could lead to a reduction in the pollen sterility of the male sterile parental clone stock due to modifications in the thickness of the anther wall (tapetum) or to reductions from one generation to the next in the inhibition of the cytoplasm accretion of the pollen.

Although the general self-incompatibility of alfalfa means that the male sterility of the clone parents is generally higher in breeding, and particularly in seed production practice, than the sterility determined microscopically, efforts must nevertheless be made to reduce the probability of self-fertilization to zero through the production of environment-stable male sterile genotypes.

The experiments aimed to answer the following questions:

(1) How is male sterility transmitted by male sterile genotypes inbred (and then crossed) to various extents in the case of open-pollination (in a fertile genotype environment unselected for sterility maintenance)?
(2) Does the male sterility of the parents have any effect on the agronomic characters of the progeny?
(3) What correlations can be observed between the open-pollination (OP) progeny characters of male steriles?

Materials and methods

The parental genotypes used in the experiment were chosen in the field in autumn 1985 on the basis of above-average open-pollinated fertilization. The male sterility of the parents was determined in summer 1985 by microscopic examinations (on the basis of 10 visual fields). Analyses were begun in the greenhouse in the winter of 1985/86 on 2×25 progeny of each maternal family, though this number later diminished (21—33 plants) due to plant destruction.

The individual green mass, plant height and shoot number of the progeny were measured at the beginning of flowering (January-April 1986). Prior to cutting, the pollen sterility was determined under greenhouse conditions.

The relation between parental male sterility and progeny characters was studied using the correlation pairs method, and the correlations between individual progeny characters by means of path analysis (Ezekiel-Fox 1970, Sváb 1973).

The general scheme of the correlations was as follows: (Figure 1).

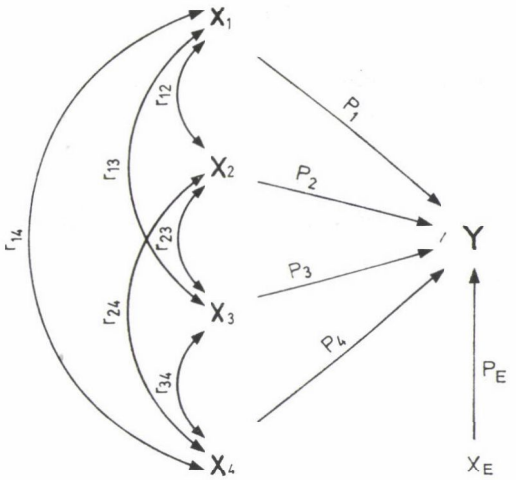

Fig. 1. The scheme of the multiple linear regression analysis. X_1 = pollen-sterility of the parents; X_2 = seed size; X_3 = plant height of the progeny; X_4 = shoot number of the progeny; X_E = error component; P_1 P_2, P_3, P_4 and P_E = path — coefficients of the characters

Table 1

Characteristics of the male sterility of parental genotypes
Kompolt, 1985

Genotype	Pollen sterility % (in the field)	Remarks
(A-SC3-16/09-22)B9-4	95.61	205 pollen grains in 10 fields of vision. 100% dehiscent sterile in the greenhouse in the 1st examination
(A-SC3-16/09-22)B9-39	100.00	No fertile pollen either in the field or in the greenhouse
(A-SC3-16)09-42	80.00	Completely sterile in the greenhouse, in the nursery 110 pollen grains (22 of them fertile) were found in 10 fields of vision
cms $BC_5109\text{-}12/_{1-4}/_{73-14}$xB13	98.21	The F_1 of a BC_5-generation cms genotype, produced with a B9 restorer and the B13 restorer. Completely sterile in the greenhouse. A total of 56 pollen grains (1 fertile) in the field
cms $BC_5109\text{-}12/_{1-4}/_{73-59}$	100.00	A stable pollen-sterile, inbred genotype
(cms AO1×S_1B9-5)-26	95.28	The F_1 of the cms genotype and a restorer progeny originating from self-fertilization. Completely pollen-sterile in the greenhouse

Average sterility of the six parental genotypes: 94.85%

Results

In the course of open-pollination, male sterility of the parents decreased by 6.25—27.88% from one generation to the next (Table 1: 2).

Table 2

Male sterility of open-pollinated progenies
(greenhouse, 1985/86)

Code	Designation of progeny	Plant No. (n)	Average male sterility (%)	$S_{\bar{x}}$	No.	Frequency of 100% sterile genotypes	Variation interval
1.	OP-cms $BC_5109\text{-}12/_{1-4}/_{73-59}$xB13	27	86.03	2.87	8	0.30	46.88—100
2.	OP-cms $BC_5109\text{-}12/_{1-4}/_{73-14}$	21	80.37	3.25	6	0.25	52.00—100
3.	OP-/A-SC3-16/09-22/xB9-39	33	79.84	2.60	4	0.12	48.48—100
4.	OP-/A-SC3-16/09-22/xB9-4	25	89.35	2.98	11	0.44	61.22—100
5.	OP-/A-SC3-16/09-42	31	88.61	2.67	9	0.29	52.38—100
6.	OP-/cms AO1xS_1B9-5/-26	21	80.25	—	9	0.43	24.52—100

Of the 6 parental genotypes examined, two were 100% male sterile, but their progeny were no better than those of 95% or 80% male sterile parents (Table 1). The renewal of fertility is also indicated by the non-significant, negative r value (—0.45). In the case of one parent, however, an increase in pollen abortion was observed even in the open-pollinated generation.

When analysing the male sterility of progeny groups, the Bartlett test showed significant inhomogeneous deviation, so the (cms A01 × S_1B9-5/-26) progeny were omitted from the evaluation. Of the five male sterile progeny groups, the 2nd and 4th, and the 3rd and 5th significantly differed from each other.

In the case of the open-pollinated progeny it can be assumed that a very large number of fertile plants made up the paternal population. Consequently, the family differences can be attributed primarily to differences in the maternal genotypes. The maternal genotype (A-SC3-16/09-42) had already attention with respect to male sterility transmittance. As regards yield analysis, the outstanding general combining ability is of especial interest. The F_1 of the Bulgarian male sterile and the Hungarian restorer had significantly better general combining ability than its BC_1 relatives (see average yields of the 3rd and 4th families) (Table 3).

In the case of the 6th treatment the Bartlett test showed inhomogeneity in the green yield deviations too, so the analysis was restricted to 5 progeny groups.

Table 3

Green yield of open-pollinate maled sterile progenies
(greenhouse containers, 1985/86)

Code	Designation of progeny	No. of plants (n)	Individual green yield (g)	$S_{\bar{x}}$	Variation interval
1.	OP-cms $BC_5109\text{-}12/_{1-4/73-59}$	27	7.59	1.03	2.0—23.0
2.	OP-cms BC_5 $109\text{-}12/_{1-4/73-14}$xB13	21	8.50	1.17	2.0—32.0
3.	OP-/A-SC3-16/09-22/xB9-39	33	5.61	0.93	1.0—19.0
4.	OP-/A-SC3-16/09-22/xB9-4	25	6.84	1.07	0.1—22.0
5.	OP-/A-SC3-16/09-42	31	11.47	0.96	3.0—23.0
6.	OP-/cms AO1x$S_1$09-5/-26	21	17.02	—	5.0—46.0

The male sterility of the parent was in negative correlation with all the progeny characters studied (Table 4).

On the assumption that the formation of abnormal pollen was partially the result of abnormalities in the cytoplasm accretion, an attempt was made to discover depression correlated with male sterility on the basis of the size of the reproductive organ (seed). The correlation analysis indicated a weak (non-significant) negative connection between the pollen sterility of the parental genotype and the seed size (length). In general, smaller seeds were produced

Table 4

Simple correlations between parental male sterility and progeny characters

		Progeny				N
		Pollen sterility	Seed size	Plant height I.	Green yield	
Parent	Pollen sterility	—0.45	—0.16	—0.76*	—0.39	6
Progeny	Seed size	0.1112	—	0.0998	—0.0950	157
	Male sterility	—	—	0.0726	0.0237	157
	Shoot No.	—0.0014	—0.1281	—0.0207	0.5088***	157
	Plant height II.	—	—	—	0.3807***	157

*, *** = Significant at the 0.05 and 0.001 levels, respectively

by the more sterile parent, and more fertile progeny were obtained from smaller seeds (an $r = 0.1112/n = 157$) correlation was demonstrated between the seed of the progeny and the male sterility of the genotypes produced from them. In contrast to previous observations and to the literary data

(Carnahan 1963, Pedersen—Hill 1972), a negative non-significant correlation was found between seed size and the green weight of 4-month-old plants in the greenhouse.

Multiple regression analysis was carried out on the effect of the progeny characters examined (seed size, progeny male sterility, plant height and shoot number at flowering) on the green yield. Of the independent variables, only the direct effects of the two yield components, plant height and shoot number, were statistically significant (Table 5). Male sterility played the

Table 5

Results of multiple regression analysis

Variable	Mean X and Y	Deviation s	Bivariate correlation	Partial			Total effects
				Regr. coeff. b_i	Regr. coeff. st. b_i	Significance	r_{yi} b_i
Seed size	2.17	0.268	−0.0950	−1.9099	−0.0700	1.11	−0.0067
Male sterility	84.80	16.387	0.0237	0.0015	0.0033	0.05	0.0001
Plant height	66.59	24.052	0.3807***	0.1210	0.3979	6.38***	0.1514
Shoot No.	5.32	2.877	0.5088***	1.2891	0.5081	8.14***	0.2585
Green yield	9.15	7.296		−1.7481 = a			0.4167

Table 6

Breakdown of bivariate correlation coefficients leading to dependent variables

X_1 (seed size)	direct	P_1	−0.0700
	indirect X_2	P_2r_{12}	0.0004
	X_3	P_3r_{13}	0.0397
	X_4	P_4r_{14}	−0.0651
			−0.0950 = r_{y1}
X_2 (male sterility)	direct	P_2	0.0033
	indirect X_1	P_1r_{21}	−0.0078
	X_3	P_3r_{23}	0.0289
	X_4	P_4r_{24}	−0.0007
			0.0237 = r_{y2}
X_3 (plant height)	direct	P_3	0.3979
	indirect X_1	P_1r_{31}	−0.0070
	X_2	P_2r_{32}	0.0002
	X_4	P_4r_{34}	−0.0105
			0.3806 = r_{y3}
X_4 (shoot number)	direct	P_4	0.5081
	indirect X_1	P_1r_{41}	0.0090
	X_2	P_2r_{42}	0.0000
	X_3	P_3r_{43}	−0.0083
			0.5088 = r_{y4}

least important role in yield potential. Seed size was also of little significance. The importance of plant height in the formation of the yield was 120.6 times as great as that of male sterility, and that of shoot number 154.0 times as great. The direct effects of the main yield components was not modified by indirect effects. Seed size had a negative effect on yield potential due to its influence on the shoot number, but the other indirect effects were non-significant (Table 6).

Conclusions

Pollen-sterile individuals were found with high frequency in the open-pollinated progeny of genotypes with a high degree of male sterility. The cytoplasm of the progeny was of two types: those designated cms BC_5 were of Hungarian origin, and those designated A-SC3 were Bulgarian. A relatively slight reduction in male sterility was indicative of female (cytoplasm) dominance. It can be assumed that those male sterile genotypes which are less inclined to become fertile again in a pollen-fertile environment will be suitable for use as parents of synthetics in order to increase the frequency of cross-fertilizations, or maintain it, during the syn-generation. Their utilization will reduce the probability of inbreeding even in synthetics made up of few components and will moderate the decrease in variability (vigour) in the course of variety reproduction.

An unexpected result was obtained in the case of the 42nd F_1 of the Bulgarian male sterile A-SC3 and the Hungarian restorer 09: the male sterility of the OP progeny was 8.6% higher than that of the maternal genotype. Since both generations (F_1 and its OP progeny) possess the same cytoplasm, it could be that the high level of male sterility was not manifested due to the inhibition of the parental genotype, while the differing genotype of the progeny led to a more favourable genotype $\times$ cytoplasm interaction.

It is very probable that it will prove easier to find a large number of restorers for male sterile genotypes which give highly sterile progeny even in the case of open pollination, thus facilitating more effective selection for other characters (yield potential, persistance, resistance etc.).

In the greenhouse container experiment, the progeny of two F_1 plants (the 5th and 6th treatments) had outstanding yield potential. According to the Bartlett test the 6th treatment had to be excluded from the analysis, as the variation interval was roughly twice that of the other progeny groups. From the point of view of breeding, however, the 17.02 g/plant yield potential is very promising and suggests that further examinations should be made.

The results of multiple regression analysis indicate that selection should be fundamentally based on the principal yield components. The experiment

provided new data to show that male sterility does not necessarily cause a depression in the yield potential.

The slight interaction between seed size and genetic yield potential was not sufficient to demonstrate greater vitality in plants raised from larger seeds. In general: A plus-variant population as regards seed size cannot be expected to have superior yield potential.

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Animal physiology and animal breeding

CHANGES IN THE BEHAVIOUR OF LAMBS

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The authors studied the ontogeny of various behaviour patterns of lambs. For five days the lambs and ewes were kept in individual then in common boxes. The observations were made of merino, racka- and awasi sheep. As regards the purpose of utilization the three breeds are rather different from one another. In spite of this the authors did not find significant differences among the breeds in the patterns studied by them.

Studying the suckling behaviour, they found that at the age of 10 days 70—80% of the lambs suckled standing by the side of the ewe. Twin lambs maintained this habit for a longer time than single lambs. The difference is significant at the age of 15 and 20 days. Up to the age of 20 days 67—80% of the twin lambs start suckling with the same teat.

On the first day the ewe recognizes the lambs exclusively by the sense of smell. Visual recognition appears on the 5th day and by the 20th day the share of visual control becomes considerable (36%). When transferred to common boxes the ewes predominantly recognized their lambs by odor. At 10 days of age in the lambs, eating and lying are common activities of ewes and their lambs. At the age of 20 days the lambs begin to wander from the ewe and become independent.

The time spent in play increases with the age of the lambs. No change in the proportions of the different elements of playing were observed by the authors up to the age of 20 days.

The authors suggest to determine the time of weaning not only on the basis of body weight, but also considering the time when the lambs form groups which indicates their independence.

Keywords: common behaviour of ewe and lamb; recognition of lambs: by odor, sight and sound; ontogeny of independent behaviour of lambs; suckling of twin lambs; play and changer in the proportions of playing elements; suckling behaviour.

Introduction

In the course of ontogeny changes occur not only in the structure of body but also in the behaviour. The changes in behaviour appear first of all in behaviour patterns which are not controlled by genetically coordinated mechanisms, though there are forms of behaviour based on hereditary mechanisms which change with experience. Ontogeny thus affects such behaviour patterns which

— either develop gradually, in response to certain stimuli,
— or require learning to develop.

With mammals the ontogeny of behaviour is not as spectacular as it is with lower animals. The bees, for example, as soon as they emerge clean the cells for the next egg laying; at the age of 14 days they show a nursing behaviour, then fulfil building tasks. From the 20th day on, they guard the entrance of the hive, then gather pollen and nectar up to the end of their lives.

In spite of the fact that the behaviour of domestic animals when young is generally known, reports on systematic investigations and evaluations of ontogeny are infrequently encountered.

Lorenz's (1985) classical observations were not made on domestic animals. At the ethological symposium held in Kiel, 1984, although the ontogeny of behaviour was on the agenda, the lectures delivered dealt with cattle, pig and poultry (Kovalcikova and Kovalcik 1984, Metz 1984).

The authors mostly studied the attachment of newborns to parents and substitute parents, the question of the critical period and the effect of the latter on growth (Hersher, Richmond and Moore 1963, Schmith, Van Toller and Boyes 1966, etc.). As for lambs, the publications mainly deal with changes in the time and frequency of eating, suckling and moving (Czakó 1978, Sambraus 1978, Bogner and Grauvogel 1984, Shillito and Williams 1986, Slee and Springbett 1986).

Materials and methods

Our observations were made of merino-, racka- and awasi sheep. As regards the purpose of utilization, these are breeds distant from one another. Lambs of these three breeds were from the beginning examined under identical conditions. For five days after birth the animals were kept in individual boxes, then the ewes and their lambs were transferred to common pens.

The 12-hour observations were made partly by means of video cameras and partly by subjective data survey. The data were processed by the statistical method.

Beyond the changes in the time and frequency of suckling, moving and lying, we studied the phenomena accompanying suckling, the relation between ewe and lamb and the socializing habits of lambs, as well as the changes in these phenomena in an early period of life, in order to find ethological basis for the determination of the time of weaning.

Results

The proportion of suckling while standing sidewards is 100% in the first days both with single- and twin lambs, and 70—80% from the 10th day (Fig. 1).

From the age of 15—20 days this way of suckling becomes less frequent; the lambs increasingly often suckle from behind, and usually utter sounds previously. Twin lambs keep the habit of suckling while standing sidewards for a longer time than single lambs. On the 15th and 20th day the difference is significant. Twin lambs also try to suckle from behind, but if they suckle at the same time they do it more comfortably from the side of the ewe. There

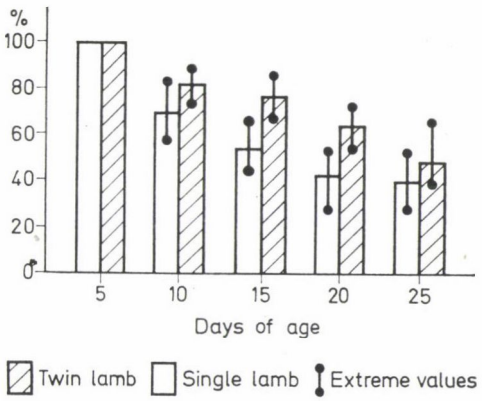

Fig. 1. Frequency of sucking from a sideward standing position in twin- and single lambs

is room between the two hindlegs for one lamb but not for two to reach the udder from behind.

Table 1 shows the trend of suckling the same teats for single and twin lambs. Single lambs generally suckle the teats of the ewe alternately. Twin lambs up to 20 days of age show preference for one of the teats, and in 67—80% of the sucklings, begin with the same teat.

Table 1

Suckling of the same teats by twin- and single lambs
(n = 28)

		Percentage of daily suckling occasions				
		5	10	15	20	25
		days of age				
Suckling of the same teat:						
twin lambs	$\bar{X}$	80.3*	76.4*	70.1*	67.2*	52.6
	± s	12.6	14.2	11.9	13.6	12.3
single lambs	$\bar{X}$	51.8	48.6	53.1	45.8	46.2
	±	13.6	12.9	14.1	10.6	11.3

* = The difference between twin- and single lambs is significant (at P = 5%)

An interesting observation concerns the habit of lambs' moving their tails when suckling (Fig. 2).

In the post-natal days the lamb when suckling raises its tail and rhythmically moves it. By the 20th day this behaviour becomes much less dominant, particularly when the lamb takes a kneeling position, although the kneeling

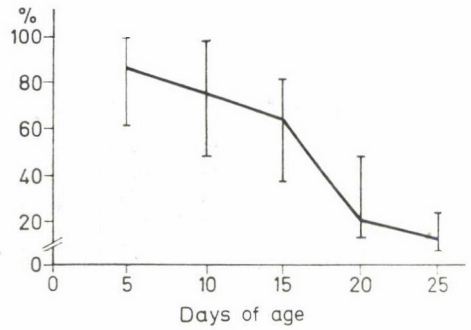

Fig. 2. Lambs' moving their tails when sucking (n = 36)

position does not prevent the lamb from moving its tail — which can be interpreted as an expression of pleasure. We do as yet know the reason for this change of behaviour.

When the lambs and ewes are placed in common pens the lambs also try to suckle from other ewes which, however, refuse these attempts. On the first and second day in the common pen these attempts are frequent (Fig. 3), but by the 5th day (with ten-day-old lambs) they practically disappear. The lambs learn that they are given milk only by their own mothers.

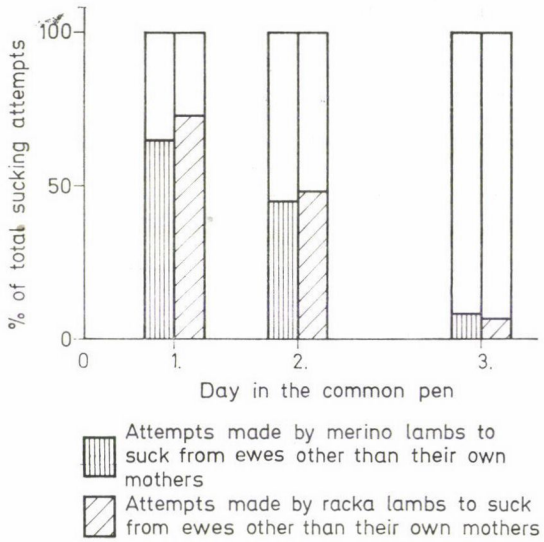

Fig. 3. Trend of suckling attempts of five- to ten days old lambs in the common pen

Table 2 shows the ways by which ewes recognize their lambs. In the individual box on the first day the ewe almost without exception (in 95%) sniffs at its lamb before allowing it to suck. On the fifth day the ewe accepts

the lamb merely by looking at it. When in the common pen smelling becomes again the main way of recognition, and it is only after 20 days that visual location begins to play any considerable role (36%). In our study the merino-, racka- and awasi ewes and lambs showed no significant differences in recognition behaviour.

Table 2

Recognition of lambs under different conditions of keeping and at different ages (n = 28)

Proportion of recognition in %		In individual box			In common pen		
		1	5	10	15	20	25
		days of age					
By odor	$\bar{X}$	95.3	63.8	84.3	67.1	27.5	16.2
	± s	6.2	8.4	5.8	10.2	4.8	2.1
By sound	$\bar{X}$	4.7	7.6	11.7	16.9	36.3	22.7
	± s	0.6	5.7	1.9	2.0	5.1	4.2
By sight	$\bar{X}$	—	28.6	4.0	16.0	36.2	61.1
	± s		5.1	0.7	6.3	7.1	10.8

Recognition by sound begins when the lamb gets out of its mother's sight. This occurs in the common pen generally at the age of 10—15 days. The ewe calls its lamb(s) repeatedly by bleating, then the lamb runs to its mother and begins suckling. When bleating has no result the ewe starts to search for the lamb. Later the situation changes: the lamb calls its mother by bleating.

The change in the behaviour of lambs can also be followed through the time spent in moving. In the post-natal period lying is the characteristic form of behaviour, but later the lamb shows more and more activity.

Table 3 contains data on joint and independent activities of lambs and ewes. At the age of 10 days, eating and lying are joint activities of ewes and lambs. Detachment from the mother begins at the age of 20 days, at which time the distance between lamb and ewe increases. If the lamb and the ewe eat at the same time the distance between them at the age of 10 days is small, because the lamb keeps trying ot eat roughage beside its mother. At the age of 20 days the distance is determined first of all by the fact that the lamb consumes fodder, the ewe hay, and the feeders are not side by side.

With lambs kept in groups, playful behaviour (running around, bouncing, pushing) appears at an early age (Fig. 4). Time spent in playing increases with age. Changes in the proportions of various elements of playing were not observed until 20 days of age. As seen in Fig. 4. lambs of different breeds spend nearly the same time at play.

Table 3

	Distribution of behaviour, %		Distance between ewe and lamb, m	
	10	20	10	20
	days of age			
Lamb and ewe lie	31.1	12.3	0.7±0.21	2.0±0.21
The lamb lies, the ewe eats	5.0	34.5	1.1±0.10	2.9±0.19
Lamb and ewe eat	61.3	21.0	0.2±0.03	1.6±0.24
The lamb eats, the ewe lies	2.6	32.2	1.2±0.31	3.2±0.17
Total %	100.0	100.0		

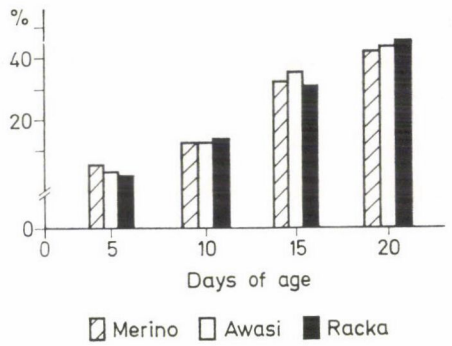

Fig. 4. Trend of time spent by lambs playing in the same observation period (n = 36)

Conclusions

The behaviour patterns are known to be hereditary, i.e. inborn, features. These hereditary forms of behaviour are accompanied by activities that change in time, such as the position taken up by the lamb when suckling, which modifies with growth.

For 2—3 weeks after birth the lambs generally suckle in a standing position. Later they gradually take up a kneeling position which is supposed to be more favourable for them. Further, from the age of 15—20 days, suckling from behind becomes more and more frequent. Twin lambs keep the habit of suckling from the side of the ewe for a longer time. Changes in the activities which accompany the process of suckling seem to be related to an increasing independence of lambs. Of course, it cannot be excluded either that a decrease in the extent of maternal care — which is difficult to express by any unit of measurement — also plays some role in these changes.

With the advance of age the expression of attachment between ewes and lambs also modifies. In the post-natal period recognition by odor is

dominant, since the contact with the newborn lambs is brought about by licking and by rubbing of muzzles; thus the faculty of recognition is linked to smells. Recognition through sounds begins when the ewe and its lamb cannot see each other. Further investigations are required to find out how long ewes and their lambs take to learn identification by sound.

According to the relevant literature, visual recognition appears two weeks after birth (Sambraus 1971, Morgan et al. 1975). Our own observations unambiguously suggest that in individual boxes the ewes recognize their lambs visually at the age of 4—5 days, as demonstrated by the fact that in some 30% of the cases the ewe allowed its five-day-old lamb to suckle without previously identifying it by odor or sound. This is only possible when the ewe recognizes its lamb. In common pens (small-group dropping) this way of identification is unusual in the first days, because lambs from other mothers also make attempts at suckling.

The stimulus exciting the playful behaviour may be highly diversified. As to its function — though the opinions vary — the play is a preparation for functions in adulthood. As to its content, it is an expression of pleasure. The time spent in playing initially increases and later decreases. This period of playfulness can be explained. At the same time, with the advance of age there is not considerable change in the proportions of the elements of playing such as running about, bouncing with stiff legs and pushing each other. Playing serves to promote the socialization.

A survey of the changes of behaviour makes it clear that they all serve the fulfilment of biological demands, the normal functioning of life and ultimately the survival of the species.

A knowledge of the change of behaviour helps in drawing some practical conclusions. For economic reasons the contact between ewe and lamb should be broken as soon as possible. Although the increase in body weight provides a certain basis for choosing the time of weaning, it is still not the main criterion. Only the signs suggesting socialization — grouping, playing together, decrease in the number of sucklings — show the time of waning, when the development of the behaviour of lambs makes them suitable for an independent life (Fig. 5). In our opinion the threefold birth weight is not enough to decide the time of weaning; the time spent by the lambs separated from their mothers and the decrease in the number of daily sucklings must also be taken into consideration.

Our earlier investigations (Czakó and Mihálka 1968) unambiguously pointed out that, in the case of lambs of good milkers, the number of suckling occasions decreased when the conditions of socialization were promoted. Social behaviour and food uptake are of determinative value in the course of ontogeny.

According to our investigations under Hungarian conditions suitability for independent life develops by the 25th and 30th day after birth.

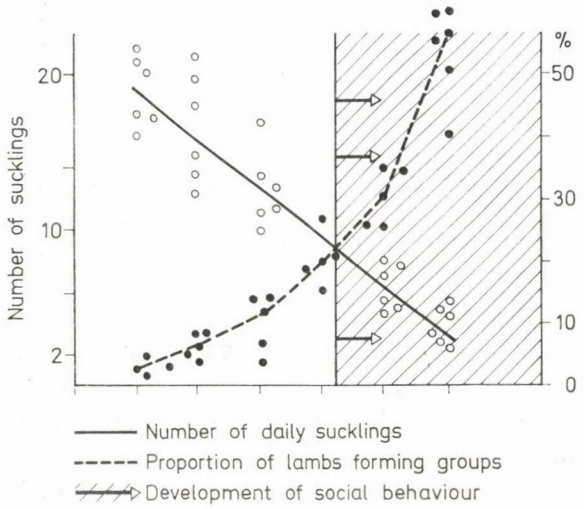

Fig. 5. Trends of sucklings and grouping in sucking lambs

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EFFECT OF THE GLUCOSINOLATE CONTENT OF EXTRACTED RAPESEED MEAL ON PROTEIN CONVERSION IN PIGS

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The authors substituted two different glucosinate type extracted rapeseed meal for 25% and 50% of extracted soybean meal, respectively, in feed mixtures for young pigs, in order to discover the changes that would take place in the protein metabolism of the pigs.

According to the result of their examinations the rapeseed meal containing 80 μmol/g glucosinate, when substituted for 50% of extracted soybean, decreased the N retention of the pigs by 16% and the productive conversion of protein by 10%. The same proportion replacement of soybean by rapeseed meal containing 128 μmol/g glucosinate decreased the N retention by 25% and the productive conversion of protein by 19%. The unfavourable protein conversion proved that in response to rapeseed fed the urea concentration of blood increased by 12% and 29% depending on the glucosinate content.

These results confirm the importance of reducing by breeding the antinutritive components of rapeseed, which is otherwise a feed of considerable protein- and relatively balanced amino acid composition.

Keywords: extracted rapeseed meal, biological value of protein, glucosinolate content, N metabolism of pig.

Introduction

In Hungary the source of protein used in fodder mixtures for pigs is mostly formed by extracted soybean meal, a large proportion of which comes from imports. For this very reason it is of great importance to seek out domestic sources of protein and incorporate them in the fodder mixtures.

As a result of an increase in the production area of soya in Hungary soybean, further such legumes as horse-bean, lupine, as well as pea, widen the range of protein sources suitable for use in concentrates. In addition to these, the extracted rapeseed meal, a source of protein, can be taken into account when it contains antinutritive components in minimum quantities.

The main cause of the antinutritive effect is the glucosinolate content detectable in rapeseed. According to the description of Anke et al. (1982), the alkyl side chains of glucosinolate contain isothio-cyanate and L-5-vinyl-2 thio-oxazolidon residua which exercise an antinutritive effect. The myrosinase enzyme present in the rapeseed splits the thiocyanate, the iso-thio-cyanate and the L-5-vinyl-2-thio-oxazolidon off the glucosinolates, and L-5-vinyl-2-thio-oxazolidon is formed through oxidation from the 3-butanyl-iso-thio-

cyanate too. There is no exact possibility for separating the effect of iso-thiocyanate from that of vinyl-oxazolidon.

The iso-thiocyanates influence the iodine uptake and iodine storage of the thyroid gland. Pigs given feed containing these compounds show lack of appetite, their body weight increase slows down, and goitre may also develop as a side-effect (Lüdke et al., 1985).

The glucosinate content in the average Hungarian autumn rapes is considerable. Since the rape growers have realized that this harmful component of the rapeseed can be minimized by breeding, in those regions of the world where the climate is favourable for rape production, better and better varieties are turned out from year to year (e.g. canola rape).

Today endeavours are made in Hungary too to introduce rape varieties bred partly abroad, partly in Hungary, in which antinutritive substances constitute the smallest possible proportions. In a study on Hungarian and foreign experiments aimed at reducing the erucic acid- and glucosinolate contents, Lukács (1988) revealed that in the autumn of 1987 on about two-thirds of the rape plantation areas on Hungary, the so-called "00" varieties (with minimum erucic acid- and reduced glucosinolate content) were sowns

Many authors (Bowland 1974; Bell 1975; Rundgren et al. 1985; Szelényi and Jécsai 1988) made comparisons of rape varieties with different glucosinolate content and found that the biological value and digestibility of rape protein, as well as its metabolizable energy content, changed as a function of the glucosinolate content. Bille et al. (1983) studied the effect of glucosinolates on the protein conversion of rats and on the weight of some of their organs, and found that the weight of the liver and the thyroid gland remarkably increased under the influence of glucosinolates.

According to Salo (1982), Näsi et al. (1985) and Baidoo and Aherne (1987), the extracted soybean meal can be excellently replaced by extracted rapeseed meal, providing that the glucosinolate content of the latter does not exceed 40—50 μmol/g.

Considering that "00" rapes of domestic production have so far been available for feeding purposes in small quantities, we thought it reasonable to investigate what changes the replacement of extracted soybean meal by rapeseed meal with various glucosinolate contents would cause in the protein metabolism of young pigs.

Materials and methods

Extracted rapeseed meal was obtained for the experiment partly from the Csepel Vegetable Oil Factory (variety: Jet Neuf, Lindora) and partly from the Csopak-Tája Co-operative Farm, Nemesvámos (variety: Tandem).

We determined the nutrient content of the samples by the MSZ 6830 standard, and their amino acid composition with a BC-200 type amino acid analyser. The glucosinolate content was determined at the Vegetable Oil and Detergent Research Institute.

The biological value and digestibility of rape protein as well as its net and productive utilization were determined in N-balance tests with young rats (Eggum, 1973).

The N-balance tests were performed with 40—50 kg young barrows in a five-day experimental phase after 12 days of pre-feeding. Before starting to feed them the experimental fodder mixtures, as well as on the last day of the N-balance, blood samples were taken from the animals. The blood was drawn in the morning, 3 hours after the consumption of 200 g fodder. From the blood the total protein content was determined by the Biuret method; the total amino acid-N content after the description of Folin and Danielson, while the urea content was measured on the basis of Berthelot's reaction. The cholesterol concentration of blood was determined according to Zlatkis and Munk (Bálint 1962).

Description of the experiments

Chemical analyses

The nutrient content and amino acid cmoposition of the rapeseed samples used for the analyses are shown in Table 1.

Table 1

Chemical composition of extracted rapeseed meals used in metabolism experiments

	Extracted rapeseed meal	
	Nemesvámos	Csepel
Dry matter content %	92.7	92.7
Crude protein content %	36.9	35.9
Crude fibre content %	10.6	10.2
Raw fat content %	1.9	2.4
Ash content %	7.1	6.9
N-free discharge content %	36.2	37.3
Total glucosinolate* μmol/g content	80.0	128.0

* Result of analysis by the Vegetable Oil and Detergent Research Institute

Amino acid composition in terms of dry matter percentage

Asparagine	2.58	2.30
Threonine	1.36	1.28
Serine	1.52	1.60
Glutamic acid	6.80	6.33
Proline	0.80	0.79
Glycine	1.54	1.51
Alanine	1.70	1.78
Cystine	0.78	0.66
Valine	1.64	1.55
Methionine	0.77	0.69
Isoleucine	1.29	1.20
Leucine	2.32	2.20
Tyrosine	1.03	0.96
Phenylalanine	1.33	1.24
Lysine	2.40	2.26
Histidine	0.90	0.90
Arginine	2.04	2.02
Tryptophan	0.14	0.16
Available lysine content *(in vitro)*	1.81	1.55

The difference in nutrient content between the two rapeseed meals was insignificant, the crude protein content being 35.9—36.9%, the crude fibre content 10.2—10.6%, the row fat content 1.9—2.5% and the ash content 6.9—7.1%, respectively.

As for the glucosinolate content, the difference between the two rapes was 60%, as the rape obtained from Nemesvámos contained 80.8 μmol/g, while the one received from Csepel 128.0 μmol/g glucosinolate.

The two rapeseeds also differed in essential amino acids. Larger quantities were measured, in general, in the sample obtained from Nemesvámos and smaller ones in that sent us from Csepel; accordingly, out of the most important amino acids cystine was 0.78% and 0.66%, methionine 0.77% and 0.69%, lysine 2.40% and 2.26%, respectively.

Experiments with animals

(1) The protein conversion indices obtained on the basis of N-balance examinations on rats are contained in Table 2. Accordingly, in the rapeseed from Nemesvámos the biological value of protein — which indicates how much of the digested protein has been converted — was 68%, while in that

Table 2

Major protein conversion indices of extracted rapeseed meals used in experiments with pigs on the basis of N-balance tests with rats

Rape variety	Protein				
	Biological value	True	Apparent	Net	Productive
		Digestibility		Utilization	
Tandem (Nemesvámos)	68	80	67	55	32
Jet Neuf — Lindora (Csepel)	63	76	63	48	21

from Csepel 63%. (For the sake of comparison we mention here that the biological value of the "00" rapeseed may reach 80%) (Szelényiné és Jécsainé 1988), and in the case of an excellent variety (Erglu) even 91% (Schulz and Petersen 1978).

The actual digestibility of protein was — in the above order — 80% and 76%, respectively.

The net utilization of protein — which expresses how much of the protein consumed has been utilized — was 55% for the Nemesvámos and 48% for the Csepel sample.

The productive utilization of protein — expressed by the ratio between the protein consumed and that retained in the organism — was 32% and 21%, respectively.

Thus, the experiments with rats suggest that, despite nearly identical protein- and lysine contents, the protein conversion in the organism decreases as a function of the glucosinolate content. However, it should be noted that according to McKinnon and Bowland (1977) rats are more sensitive than pigs to the glucosinolate content of the rapeseed.

(2) In metabolism experiments with young pigs the extracted soybean was replaced to 25% and 50% respectively by seed meal of the two mentioned rape varieties (Table 3). Group 1 was used for control and the feed for them

Table 3

Composition and nutrient content % of fodder mixture in metabolism experiment with pigs

Denomination		1.	2.	3	4.	3.
		group				
Maize		40.00	35.00	30.00	35.00	30.00
Wheat		43.90	48.90	53.85	48.90	53.85
Extracted soybean		12.00	9.00	6.00	9.00	6.00
Extracted rapeseed (Nemesvámos)		—	3.00	6.00	—	—
Extracted rapeseed (Csepel)		—	—	—	3.00	6.00
L-lysine		0.10	0.10	0.15	0.10	0.15
Premix MK-1-2/F		4.00	4.00	4.00	4.00	4.00
		100.00	100.00	100.00	100.00	100.00
Dry matter content	%	87.5	87.4	87.4	87.5	87.5
Crude protein content	%	15.1	15.0	15.5	14.9	14.7
Raw fat content	%	2.6	2.5	2.4	2.7	3.1
Crude fibre content	%	3.2	2.9	3.9	2.7	3.5
Ash content	%	3.2	3.1	3.2	3.2	3.1
Lysine content	%	0.71	0.70	0.72	0.70	0.72
Digestible energy content	MJ/kg	13.7	13.8	13.4	13.9	13.6

contained 12% soybean. In groups 2 and 3, rapeseed from Nemesvámos, while in groups 4 and 5, rapeseed from Csepel was substituted for soybean in the feed. Each group was given lysine supplement according to need.

In our experiment the crude protein content was 14.7—15.5%, the crude fibre content 2.7—3.9% and the lysine content 0.70—0.72%, and the energy content 13.4—13.9 MJ/kg, respectively, in the fodder mixtures (Table 3).

The protein conversion values of the fodder mixtures for pigs were determined in a N-metabolism experiment with rats (Table 4). Accordingly, while the biological values of the soybean control and the fodder mixtures containing rapeseed from Nemesvámos were nearly identical (79% and 81%, respectively), the fodder mixture prepared with the higher glucosinolate content rapeseed from Csepel was found to be of decreased biological value

(77% and 74%, respectively). The same tendency can be seen for the digestibility of protein as well as for its net and productive conversion.

The results of the N-metabolism experiment with pigs can be seen in Table 5.

Table 4

Major protein conversion indices of fodder mixtures for pig on the basis of N-turnover tests with rats balance

Group	Protein feed	Protein				
		Biological value %	True	Apparent	Net	Productive
			Utilization %		Digestibility %	
(1)	Extr. soybean	81	89	75	72	48
(2)	3% rapeseed (Nemesvámos)	79	90	77	72	49
(3)	6% rapeseed (Nemesvámos)	80	87	74	70	46
(4)	3% rapeseed (Csepel)	77	86	73	67	43
(5)	6% rapeseed (Csepel)	74	86	73	64	41

Table 5

Replacement of extracted soybean meal by various extracted rapeseed meals in N-metabolism experiment with young pigs

Group	Protein feed	N-intake g	N-excretion		N-balance g	N-digestibility g	Productive protein utilization g
			in urine g	in faeces g			
(1)	Extr. soybean	33.92	8.80	3.79	21.33±1.8	88.8±1.1	62.9±5.5
(2)	3% rapeseed (Nemesvámos)	32.86	9.92	4.12	18.82±3.8	87.4±3.2	57.3±11.5
(3)	6% rapeseed (Nemesvámos)	31.69	10.00	3.70	17.99±1.8	88.3±2.4	56.7±5.9
(4)	3% rapeseed (Csepel)	33.25	11.36	4.47	17.42±1.8	86.5±3.0	52.4±5.5
(5)	6% rapeseed (Csepel)	31.79	12.16	3.52	16.11±1.5	88.9±0.6	50.7±4.8

The quantity of N taken up from the feed ranged between 31.69 and 33.92 g, The lower N uptake occurred in those cases when 50% of the soybean was replaced by rapeseed (groups 3 and 5). The quantity of N discharged in the urine was smallest (8.80 g) in the group given soybean and largest (12.16 g) in those consuming rapeseed from Csepel. The amount of N discharged with the faeces did not show such an unambiguously increasing tendency.

According to the results of the N-metabolism experiments, the N-balance was best (21.33 g) in the control (soybean group). On the contrary, a considerable decrease was observed (18.82 g) even when 25% of the soybean protein was replaced by rapeseed meal containing 80 μmol/g glucosinolate. Repeseed containing 128 μmol/g glucosinolate — when substituted for 50% soybean (group 5) — reduced the N-balance to 16.11 g.

The productive utilization of protein decreased from the 62.9% value of the control to 56.7% in the 3rd and to 50.7% in the 5th group.

Before starting to give the experimental feed, and at the end of the N-balance test we took blood from each pig. The results of the blood tests are shown in Table 6.

Table 6

Analysis results of blood samples taken from young pigs in the course of N-metabolism examinations

Group	Protein feed	Experimental feeding			
		before at the end			
		Total protein g/l	Total-amino acid-N μmol/l	Urea μmol/l	Cholesterol μmol/l
(1)	Extr. soybean	70.1±6.8	7.77±0.38	5.78±0.25	4.60±0.79
(2)	3% rapeseed (Nemesvámos)	67.0±4.2	7.47±1.23	5.79±0.20	4.33±0.53
(3)	6% rapeseed (Nemesvámos)	71.9±4.1	7.82±0.60	5.76±0.43	4.28±0.21
(4)	3% rapeseed (Csepel)	72.2±1.0	7.40±0.84	6.03±0.19	4.48±0.19
(5)	6% rapeseed (Csepel)	71.5±2.6	7.33±0.42	5.87±0.22	4.88±0.39

Before the experimental feeding the total protein content of the blood wsa 70.1—72.2 g/l, except in group 2, where it was 67.0 g/l. By the end of the experiment nearly identical values — 68.2—69.8 g/l — were obtained.

The total amino acid-N-content of the blood was 7.33—7.82 μmol/l before the experiment, and 6.44—7.58 μmol/l when it was completed.

The urea concentration of the blood was 5.76—6.03 μmol/l before the experiment started and 5.6—7.59 μmol/l when it was over.

The initial cholesterol content of the blood was 4.28—4.88 μmol/l; by the end of the experimental feeding it rose to 4.64—5.12 μmol/l.

Conclusions

The present results confirm those which we established in an earlier experiment with rats (Szelényi-Galántai and Jécsai 1988) that is, the protein conversion decreases in proportion with the increase in the glucosinolate content of the rapeseed.

The results of the N-metabolism experiment with pigs prove that when the extracted soybean is replaced by high glucosinolate content rapeseed, the N retention decreases. This decrease is proportionate to the increase in glucosinolate content. In our experiment the rapeseed containing 80 μmol/g glucosinolate decreased the N-balance by 16% while that containing 128 μmol/g glucosinolate by 25% compared to the control, in those cases when it was substituted for 50% of the soybean meal. The productive utilization of protein in the same order was decreased by 10% and 19%, respectively.

According to the data of the metabolism experiments with pigs, the use of rapeseed meal hardly changed the apparent digestibility of N.

The changes taking place in the urea concentration of blood clearly reflected the decline of protein conversion. In the case of a 50% replacement of soybean, the average values rose by 12% in group 3 and by 29% in group 5, compared to the initial values.

The unfavourable effect of the glucosinolate content of the rapeseed meal on the protein metabolism of pigs makes it reasonable or even necessary to persuade the growers to take the antinutritive components into consideration when choosing the rape varieties. A possibility is offered thereby to use rapeseed in the fodder mixtures for pigs as a component of full value, owing to its relatively favourable amino acid composition.

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Acta Agronomica Hungarica, 39 (3—4), pp. 445–451 (1990)

Book reviews

Of the publication *Beiträge zur Tropischen Landwirtschaft und Veterinarmedizin.* Karl-Marx University, Leipzig, GDR. Editor: DR. PROF. GÜNTHER FRANKE.

The 13 scientific papers contained in the publication — by German, Nigerian, Egyptian and Cuban authors — supply interesting information on the most diversified areas of tropical agricultural research.

The first 3 papers deal with questions of agricultural economics; then after 7 papers on crop production and soil conservation 3 articles discuss problems of animal farming under the conditions of developing countries.

1. *H. Drabner* (GD) shows the importance and development possibilities of nomadic animal breeding in East-Africa from the standpoint of agricultural economics.

2. *N. O. A. Ezeh* (Nigeria) recounts a survey made in the Nigerian Rivers State of the rate and type of the labour force employed by farms growing manioka and maize. He found — in opposition to the prevailing opinion — that they employed primarily wage-workers in the manioka fields, mainly for hard work. Family members only worked in the maize fields. Characteristic is the distribution of the labour force by both sex and age. The unsolved social and technological problems hinder the introduction of mechanization.

3. *D. Rudert* (GDR) summarizes the methodology of elaborating technological plans for crop production farms in developing countries. He emphasizes that the main task is the quantitative and qualitative determination of the production processes for which he also makes concrete suggestions.

4. *P. Glanze* (GDR) examines the technological elements of the production of manioka, an easily mechanizable crop, with a view to increasing the efficiency of mechanization.

5. *R. Hoffmann* and *W. Maibaun* (GDR) determined the dynamics of the mineral N forms of various tropical soils under laboratory conditions, studying the mechanisms stimulating or inhibiting the nitrification.

6. *R. Relova* (Cuba)–*J. Pohlan*–*G. Franke* (GDR) tells the effect of weed killing in different periods on the development of young caffee plantations under Cuban conditions.

7. *J. Pohlan* (GDR), *F. de la OSA*, *A. Ramirez* (Cuba), in their paper similar to the subject of the former one, discuss the effect of various methods of weed killing on the dynamics of weed association in a Cuban citrus plantation.

8. *A. F. Radi*, *M. A. Shaddad*, *A. M. Hamada* (Egypt) treated field crops with varying concentration solutions of auxins of herbicide origin and studied their effect on the cation circulation of plant parts.

9. *N. Chiejina* (Nigeria) deals with the effect of Telfairia mosaic virus in the case of four tomato varieties. The author found that the harmaful effect of virus infection on the quantity and quality of yield depended on the development stage (age) of the plants at the time of the infection. The variety "Chef" proved the most resistant.

10. *F. Wehner* (GDR) describes the profilactic protection of cotton plantations from

mite infestations, with special regard to the role of the weed flora.

11. *G. Flachowsky et al.* (GDR) evaluate the role of rice straw as a feedstuff for ruminants, and point out that owing to the high lignin content the digestibility of the rice straw is very poor, so its nutritive value is negligible, nevertheless, it plays an important role in feeding ruminants among rice-growing regions.

12. *A. Essien* (Nigeria) studied the environmental factors influencing the egg production of imported poultry breeds under humid tropical conditions, with the view of elaborating the optimum local breeding technology.

13. *El Sayas et al.* (Egypt) studied the effects of photoperiod and temperature on the production of layer hybrids and broiler chickens under subtropical conditions.

At the end of the publication, after these articles, several recently published agricultural works are briefly reviewed.

The papers of the publication give a comprehensive view of the current problems of agricultural research in the tropics, and of the latest works of literature on the subject.

J. VARGA

RAFAEL PALACIOS and DESH PAL S. VERMA: *Molecular Genetics of Plant-Microbe Interactions* 1988. (Proceedings of the 4th International Symposium on Molecular Genetics of Plant-Microbe Interactions. Acapulco, Mexico, May 15–20, 1988.) American Phytopathological Society Press, St. Paul, Minnesota, USA.

This book presents the Proceedings of the Fourth International Symposium on Molecular Genetics of Plant-Microbe Interactions that took place in Mexico, 1988. The Symposium was organized by the American Phytopathological Society and sponsored by the National University of Mexico. About 400 scientists from 25 countries participated in the meeting. Six sessions included 40 oral presentations and 200 papers were presented in the form of posters.

This book has been reproduced directly from type-written copies of 108 articles. There are grouped into five sections, such as,

(1) Recognition and specificity

(2) Signal exchange and metabolic interactions

(3) Symbiosis

(4) Pathogenicity

(5) Plant Genetics

Section I, titled Recognition and Specificity, includes 17 lectures on the ion channel defence model, molecular and genetic analysis of hew symbiotic genes in Rhizobium meliloti and the host range genes of R. trifolii or the characterization and role of polysaccharides and lipopolysaccharides.

Section II is the longest part of the book, containing 27 articles. Recent developments on organization, regulation, control and mapping of genes involved mainly in modulation (nodD, nodH and nodABC) and nitrogen-fixation (nif and fix genes) are discussed.

In Section III, 17 lectures deal with the topic of symbiosis. The first article, titled Overview of Symbiosis, offers a complete review of several interesting and obvious features of the *Rhizobium-Legume* symbiosis, and raises important questions relating to various events in symbiosis and recent developments in the relevant literature. Genes of different species, e.g. *Rhizobium meliloti* (fix), *R. trifolii* (ndv), *R. phaseoli* (sym), *R. leguminosarum* (sym and HUP), *R. fredii* (pectate lyase) as well as *Azospirillum brasilense* and *Azorhizobium caulinodans* are analysed, isolated and studied.

Section IV, the second longest part of the book, contains 28 articles concerning the molecular genetics of host-pathogen interactions. Different pathogenicity genes of *Pseudomonas syringae* and *P. solanacearum*, *Magnaporthe grisea*, *Ustilago maydis*, *Erwinia casstorora* and *E. chrysanthemi*, *Fulvia fulva*, *Cochlibolus heterostrophus*, *Xanthomonas campestris*, *Colletotrichum gloeosporioides*, tomato ringspot virus and potato virus Y are identified, isolated and analysed. Five articles concern the plant genetic engineering by *Agrobacterium tumefaciens* and its T-DNA transfer to dicotyledonous and monocotyledonous plants.

Finally, *section V* includes 11 lectures that deal mainly with plant genetics. Most of the articles here are devoted to the characterization and expression of nodulin genes in various plant species, e.g., soybean, alfalfa, bean and yellow lupin.

All in all, this book excellently summarizes the organization and expression of microbial and plant genes, as well as the nature of signals that participate in the communication between both partners in symbiotic and pathogenic interactions. It discusses the latest results and problems of the regulatory circuits and the function of gene products. The rich material of the book offers substantial help in basic and applied research on molecular-, microbial- and plant genetics, thereby promoting the further development of this field of plant-microbe molecular biology.

L. HESZKY

Scientia Agricultura Bohemoslovaca. Vol. 21. No. 2. 1989

This quarterly journal published in both the English and Russian language by the Institute for Agricultural Technico-Scientific Information of the Czechoslovakian Academy of Agricultural Sciences describes current results attained in the field of agricultural and sylvicultural research. The scientific articles reflect well the high level of today's Czechoslovakian research in applied biology. The papers contained in the present number similarly represent the areas with nowadays command particular interest in Czechoslovakia.

In all countries that grow winter cereals, winterhardiness is characteristic long studied by the producers and breeders of cereals. As Prásil and his collaborators point out, owing to the considerable differences in cropyear and testing methods, the categorization of wheat varieties for winterhardiness remains an unsolved problem worldwide, but the statistical method elaborated by the authors moves toward a solution. Wheat varieties with different frost resistance are placed in eight groups on the basis of a winterhardiness index, and the standard deviation is established. This method has the advantage that the results of experiments with different variety composition, and in winters of varying temperatures, can be fairly compared.

Machán uses oats to show Hinkelmann's asymmetrical incomplete diallel method in determining the general and specific combining ability. In his opinion this genetic analysis provides especially useful information to breeders, since the supposedly less useful combinations are not necessary to determine the genetic parameters.

The protein components demonstrable by electroforesis, e.g., the wheat gliadins, have become widely used genetic markers. Sasek and Sykorova describe an improved method of the starch-gel electroforesis elaborated by Sozinov and Poperelja; with this modified technique they characterize the gliadin blocks of the most widespread Czechoslovakian wheat varieties.

Six papers deal with research in the field of livestock farming. Antal et al., studying the fattening of Pinzgau cattle, found that 500 kg was the optimum live weight in which the increase of body weight and the quality parameters of the meat were the most satisfactory. Daily body weight gain was also studied by Pavlik et al. with Duroc and Belgian Landrace pigs. The best parameters were shown by the F1 population of these two breeds. As well as the daily weight gain, the hybrids gave the best results in feed conversion. Zazimalova et al. studied the genetic background of pig breeding, and similarly indicated the advantages of hybrids. At the same time, they stressed the indisputable importance of the synthetic lines as another essential source of genetic variability.

Results of feeding experiments with broiler chickens are reported by Chrappa et al. An important role may be played in feeding by the three-electrode portable hygrometer that Cech and Polednicek devised to maintain the quality of preserved feedstuffs.

This journal includes the 1988 list of those rewarded with the Young Researchers' Prize by the Czechoslovakian Agricultural Academy and the Youth Organization. The publication emphasizes that the works of the winners are

characterized by great theoretical knowledge and direct applicability in practice. I think, the ars poetica of the Scientia Agriculturae Bohemoslovaca can similarly be summarized with these two features.

Z. BEDŐ

SAETTLER, A. W., SCHAAD, N. W. and ROTH, D. A. (Ed.): *Detection of bacteria in seed and other planting material* 1–122. APS Press, The American Phytopathological Society, St. Paul, Minnesota 1989. — ISBN 0-89054-098-5.

One of the important factors of seed quality is its health condition, thus it is highly important to learn how to detect the seed-borne bacterial pathogens of plants. The same applies to vegetative propagation materials.

The book is divided into four chapters. The first describes the phases of examination. They are: detection of the pathogen in the seed or the vegetative plant part, isolation of the pathogen, and identification of the bacterium species.

The remaining chapters deal with the detection of various bacterial pathogens in seed and vegetative plant parts. The more important pathogenic bacteria of the major plant species are discussed, which are:

Bean: *Xanthomonas campestris* pv. *phaseoli*, *Pseudomonas syringae* pv. *phaseolicola*.

Tomato: *Clavibacter michiganense* subsp. *michiganense*, *Pseudomonas syringae* pv. *tomato*, *Xanthomonas campestris* pv. *vesicatoria*.

Carrot: *Xanthomonas campestris* pv. *carotae*.

Cruciferae, mainly cabbages: *Xanthomonas campestris* pv. *campestris*.

Protato: *Erwinia carotovora*, *Erwinia chrisanthemi*, *Clavibacter michiganense* subsp. *sepedonicum*.

Woody plants: *Agrobacterium* species.

The authors describe detailed, easily reproducible methods for the detection of the various bacterium species, and illustrate the course of detection in figures. Details are given of the composition of culture media and of the chemicals and tools required for the examinations. The literary references and the rich bibliography facilitate further detailed studies of the subjects.

The 18 black-and-white and 28 colour photographs give a clear idea of the methods of bacterium detection.

The book is indispensable for those who perform laboratory analyses of the seed-borne bacterial pathogens of plants. The detailed methodology of the book may also a useful basis for the detection in the seed of those plant pathogenic bacterium species that are not included in this book.

M. GLITS

Year-book of the Bavarian Foederative Livestock Farming Institute, Grub, 1988. Bayerische Landesanstalt für Tierzucht Grub. Jahresbericht, 1988. Band 28.

In the preface of the present 28th volume of the year-books Dr. Paul Hoffmann, President of the Institute, outlines the objectives and major tasks of its work. Besides the continuous increase of production and economic efficiency, the improvement of the quality of animal products and co-operation in the work are considered important tasks. The development and testing of the most up-to-date technologies are indispensable. Great emphasis is laid on the modernization of the performance tests and their wide application in the work of selection. The evaluation of the results is helped by their developed instruments, including a high-capacity computer centre. The Institute plays an important role in practical consultation, in professional training and in the extension training of foreign scholarship-holder researchers.

The President of the Institute is assisted in his management work by a secretariat and a publication-education group furnished with a computer centre. They have seven departments in which three to seven research teams work on solving of the different tasks. The cattle-, pig-, sheep-, and small animal farming, feeding and preservation, keeping technology, veterinary health and quality testing

departments are led by highly qualified, professionally acknowledged departmental heads.

The year-book provides brief information about the tasks and research subjects of the departments, and an account is given of studies on fallow-deer ethology as well as on biogas production and -utilization.

In the course of the Grub Institute and its research stations, 13 training courses were given with 192 participants. They took 2–95, mostly 10–25 days each. Besides, from 14 countries — mostly from the Middle- and Far East, though also from North- and South-America and Europe — 22 researchers and 19 assistants, including Hungarians, were accepted for extension training. Professional consultations and surveys — 180 in number — were held with nearly 5000 participants. The Agricultural Minister of Tanzania, as well as the Belgian, Danish, English, West-German, Irish, Swedish, Swiss and South-African Agricultural Attachés accredited to Bonn were special visitors of the Institute.

Progeny testing for meat production capacity covering some 40,000 offspring and brothers and sisters of excellent stock bulls and boars was carried out. Extensive investigations were made with bull-raising cows, broiler- and layer hybrids, and rabbits.

The Members of the Institute published 124 scientific papers in 1988. To date they have written 22 books, mostly on the farming of cattle and pigs.

I. HEROLD

HANS JOACHIM FIEDLER: *Bodennutzung und Bodenschutz* (VEB Gustav Fischer Verlag Jena 1990). (Soil Utilization and Soil Conservation)

The author of the book, Hans Joachim Fiedler, professor of Soil Science and Forestry, has published several books during the last three decades related mainly to soil fertility and to the problems of fertilizer's application. Recently, he has investigated environmental problems, and in 1987 his book appeared on the role of the trace elements in the environment. In this new book, published by VEB Gustav Fischer Verlag, he comprises numerous topics related to the functions of soils in terrestrial ecosystems with particular regard to the human environment.

After the introductory first chapter, the second characterizes the soil as part of the terrestrial ecosystem. Here the author summarizes the basics of soil science including soil morphology, soil classification, pedon description and some physical and chemical soil properties.

In the same chapter the interrelations between soils and respective ecosystems are also described.

The next chapter is devoted to the question of soil fertility, Information is provided here mainly on the pH conditions, plant nutrient contents and biological properties of soils. The technology of the application and the influence of the organic and mineral fertilities on the soil properties and soil fertility are also characterized. The regulation of soil acidity as well as the improvement of physical properties of soils are particularly introduced. The same chapter includes a few problems of irrigation. In the fourth chapter, which deals with the utilization of soil resources, the particular problems of forestland and cropland are separately described. As related to soil resources, mainly the area of the German Democratic Republic is described in this chapter with an indication of the changes in territory and branches of cultivation from 1950 to 1986.

Soil cartography and soil evaluation are the main topics of the fifth chapter. After a short description of the basic methods of the cartography of soils, the soil types and soil evaluation in the GDR are described with particular regard to its forestry.

One of the most timely and remarkable chapters of the book, the sixth, concerns the carrying capacity of the soils. Different pollutions and agents are described which affect the soil resilience, which are mechanical, hydrological, chemical thermal and biological influences. The author particularly describes the influence of tillage, precipitation and radiation as well as irrigation on soil proper-

ties and soil fertility. The influence of radioactive fission products is included here. This chapter also deals with soil contamination by trace elements emitted from industrial plants and by sludge. Increasing soil acidity and alkalinity, as results of contamination are not only described, but the rate and dynamics of the processes are also characterized and analysed by physico-chemical patterns and equations.

The threshold values of heavy metal's concentration in respect to plant tolerance are included and described in this section. Due attention is paid to several practical problems, such as the accumulation of nitrates in soil layers and in ground waters, appearance of harmful microorganismus, vectors in soil, etc.

The next chapter concentrates on similar concerns, namely the contamination of soils through application of mineral fertilizers, sludge, organic by-products and others. The problems of recycling, and the methods of protection of soils against adverse effects, are also examined. The data on the alteration of several soils effected by fertilizers and herbicides are particularly valuable in order to characterize the resilience of soils in intensive agriculture.

Besides the influence of fertilizers on soils, the effects of motorization, oil residue and sludge are also set forth, based on long-term observations. Questions of the purification of sewage are also discussed in this chapter and various methods as well as the technology are described.

The eigth chapter discusses the rehabilitation of land disturbances, with particular regard to the land degradation and deterioration caused by mining. Practical measures are also included for the reclamation of waste land and its conversion to agriculture.

The final chapter embraces practically all the recommendations related to the previous chapters in order to elaborate a comprehensive system for land, water and soil conservation in a practical and modern technique for environmental management. The system includes data basis, monitoring and even legal aspects in the framework of production and management.

Hans Joachim Fiedler has presented a valuable and up-to-date treatment of pressing problems related to our soils and environment. He provides a useful handbook not only for soil scientists and managers but also for representatives of many other branches of science as well as for laymen.

I. SZABOLCS

BRUCE MACDONALD, 1989: *Practical woody plant propagation for nursery growers*, Volume 1.

Published by: Timber press inc. 9999 S. W. Wilshire, Portland, Oregon 97 225 USA, ISBN 0-88192-062-2. 660 pages, 627 black and white photographs, 30 line drawings, numerous tables and charts.

Plant propagation is a hard job and it is especially true for the woody plants. For success, one needs a thorough knowledge of plant physiology, a good acquaintance with both the traditional and the most up-to-date equipment, and the necessary skill to handle the most arduous operations.

This handbook is the most recent and most comprehensive publication in its field. As the first volume of the scheduled 2-volumes series, it discusses in detail the principles and techniques of: Sexual Propagation (from seed), and different ways of Asexual (vegetative) Propagation, such as Cuttings (hardwood, semi-hardwood, softwood) Layering, Grafting, Micropropagation and more.

Separate chapters are devoted to the necessary facilities, media, tools, containers and diseases. In addition, numerous appendices provide lists of,

— trade names and composition of related chemicals, equipment and other materials, together with their suppliers,

— examples of wide number of species to which each technique, tool, etc. is particularly well suited,

— institutions, societies or other establishments involved in the propagation and breeding of woody plants.

The author, previously a nurseryman, himself, than a Senior Lecturer at Hadlow

College of Horticulture and Agriculture, Kent, England, now the Director of The Botanical Garden at The University of British Columbia, and the Vice President of the Western Region of the International Plant Propagators Society, has wide practical experience and personal contacts with plant propagators worldwide.

His book is oriented to the needs of the professional nursery grower, but is also a treasury of practical information for scientists.

We can eagerly look forward to the second volume, planned as a dictionary for propagation of the individual genera and species.

G. SCHMIDT

REVIEWERS OF MANUSCRIPTS, VOLUME 39, 1990

Every scientific contribution in Acta Agronomica Hungarica is reviewed by two scientifically qualified persons. The Editorial Board is pleased to publish the following list of reviewers for the manuscripts of the 1988 issues, who by their unselfish contribution have significantly contributed to ensure the scientific standards of Acta Agronomica Hungarica.

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GENERAL INSTRUCTION

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Manuscripts in English or in Hungarian including Abstract, References, Tables and Legends should be typed double-spaced (25 lines, 50 characters per line including spaces) and supplied with authors' names, page number. Tables should be on separate, numbered pages after the References. Legends for figures, on a separate page, should follow the tables. Standard articles should not exceed seven pages.

FORMAT

Title. The title should reflect the most important aspects of the article, in a preferably concise form of not more than 100 characters and spaces.

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